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ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE FORTIETH ANNUAL MEETING OF THE SOCIETY, PITTSBURGH, PENNSYLVANIA DECEMBER 6 TO 8, 1948

A new glasshouse tomato variety, of Globe type, resistant to Fusarium oxysporum f. lycopersici race 1. ALEXANDER, L. J. In 1940 Bohn and Tucker described an immunity type of resistance to the Fusarium wilt disease of tomato. This resistance was inherited as a simple dominant factor. Several of the advanced generation lines of Bohn and Tucker were used as resistant parents in the present breeding project. Parents of one cross were Globe and line M-8-2, a resistant line of the following pedigree: Earliana × *Lycopersicon pimpinellifolium* (Missouri Accession 160) × Break O'Day × Ponderosa, field selfed, × Greater Baltimore, and field selfed twice. "Ohio Wilt Resistant Globe" resulted from crossing M-8-2 with Globe and backcrossing twice to Globe. The vine characteristics of Ohio W-R Globe closely resemble those of Globe, but the fruits tend to be slightly flatter and slightly smaller than those of Globe. The quality of the fruit equals or exceeds that of Globe. The yield of Ohio W-R Globe exceeded that of Globe in the spring crop of 1946, 1947, and 1948, and the fall crop of 1946. In 1945 Alexander and Tucker demonstrated the existence of physiologic races of the pathogen and described a new race. The distribution of the new race appeared to be limited to a small area in Ohio. Ohio W-R Globe is susceptible to this race (2) of the pathogen.

Genetics of resistance to the common bean mosaic virus (bean virus 1) in the bean (Phaseolus vulgaris L.). ALI, MOHAMED A. K. Crosses were made between the susceptible variety Stringless Green Refugee and three resistant varieties, U.S. No. 5 Refugee, Idaho Refugee, and Robust, in all possible combinations. Results of inoculating F_2 plants indicate that resistance of U.S. No. 5 Refugee and Idaho Refugee is controlled by one dominant gene, while resistance of Robust is governed by a recessive gene. The results of reciprocal crosses were alike throughout. Robust × U.S. No. 5 Refugee gives in F_2 the ratio 13 resistant: 3 susceptible, suggesting a 2-gene difference. Using an approach-graft inoculation technique this ratio was broken down into 9 top necrotic : 4 resistant : 3 mosaic. Since top necrosis is characteristic of U.S. No. 5 and Idaho Refugee, but not of Robust, the results support the following factorial hypothesis: A basic dominant gene *A* is required for susceptibility, and another dominant gene *I* inhibits mosaic symptoms but gives top necrosis upon graft inoculation. Therefore, the genetic constitution of Stringless Green Refugee is *AAii* (susceptible), that of U.S. No. 5 Refugee and Idaho Refugee is *AAII* (dominant resistance and top necrotic reaction), and that of Robust is *aaii* (recessive resistance and no top necrosis). This explanation accounts for the two types of resistance and the distributions obtained from crosses between the varieties tested.

Rhizoctonia solani, a destructive pathogen of Alta fescue, smooth brome grass, and birdsfoot trefoil. ALLISON, J. LEWIS, HELEN S. SHERWIN, IAN FORBES, JR., AND ROBERT E. WAGNER. *Rhizoctonia solani* causes a disease of Alta fescue lawns and is a limiting factor in the establishment and persistence of smooth brome grass and birdsfoot trefoil in pasture mixtures at Beltsville, Maryland. During the summer seasons of 1947 and 1948, this fungus caused severe damage to spaced plants of fescue and brome grass in nurseries and to turf plots of these grasses in pure stand and mixtures. Trefoil in pure stand and mixtures was also severely attacked. A conspicuous leaf spot symptom was produced on the grasses, and infected plants were weakened and frequently killed outright. Surviving plants of the grasses and trefoil had some ability to recover. Damp, humid, warm weather favored disease development and dense, heavy vegetative growth was most susceptible to invasion by the fungus. The fungus mycelia grew rampant on all foliage parts and infection took place at random. Certain pasture grasses and legumes, orchard grass and Ladino clover particularly, had some resistance to natural infection with the fungus.

The Fusarium wilt of cowpeas and soybeans. ARMSTRONG, G. M., AND JOANNE K. ARMSTRONG. Observations of a few varieties of soybeans and numerous varieties or breeding lines of cowpeas in a field plot, as well as inoculation of these in the greenhouse with Fusarium-wilt isolates from both hosts, indicated that there are definitely two and probably other biological races on the basis of pathogenicity. Race 1 obtained from both soybeans and cowpeas caused wilting of some varieties of both hosts; while race 2 obtained only from cowpeas, caused severe wilting only of some varieties of this host. The cowpea varieties, Lady Finger and Sumptuous, served as differentials for separating races 1 and 2. Brown Sugar Crowder was susceptible, and Brabham and Iron generally were resistant to both races. No significant cultural or microscopic differences could be detected between the races, although the isolates represented a wide range of cultural types. Some isolates lost pathogenicity for soybeans but not for cowpeas. Inoculation experiments with one

or more isolates of both races 1 and 2 on the following plants failed to produce external symptoms of wilt: cotton, okra, coffeeweed, tobacco, sweet potato, mimosa, crotalaria, snapdragon, garden pea, pigeon pea, alfalfa, and hairy vetch.

Seed treatment tests with water-planted rice. ATKINS, J. G. JR., AND PAUL BOUCHEREAU. In preliminary tests in 1948, rice was treated with several fungicides and planted in water. Three field tests were made in March and April. The slurry method was used, except with Yellow Cuprocide. Yellow Cuprocide gave an average increase of 53.8 per cent in emergence over nontreated seed. Emergence was not increased by Arasan SF (tetramethyl thiuram disulfide), the standard fungicide in Louisiana for drilled rice, or by Arasan SF plus two stickers, 31X and P.e.p.s. Dow 9B wettable (a trichlorophenate) drastically reduced emergence. Yield differences between nontreated, Yellow Cuprocide, and the Arasan SF treatments were small. The effectiveness of fungicides on presprouted, water-planted rice was also studied, using pots filled with soil from rice fields. Yellow Cuprocide, red copper oxide, copper carbonate, and Tribasic copper sulphate increased emergence. In potted rice plants there was some inhibition of root development by the Cuprocide treatment. No inhibition was observed in field tests.

Western wheat mosaic in Colorado and its transmission by the grain aphid Toxoptera gramineum. ATKINSON, R. E. In 1947 an epidemic of wheat mosaic occurred in three wheat-growing districts of eastern Colorado. In 1948 the disease was generally less severe but was an important source of loss in two other districts. The virus nature of the disease was established by inoculating susceptible plants with filtered extracts of diseased plants. In repeated experiments plants grown in soil from infected fields have shown no mosaic symptoms. Experiments to ascertain if the virus is seed borne were negative. In the greenhouse, western wheat mosaic was transmitted by the grain aphid, *Toxoptera gramineum*. Single viruliferous aphids were isolated and implanted on pots of 10 healthy wheat plants. In every pot, 30 to 50 per cent of the plants had mosaic symptoms. Non-viruliferous aphids failed to produce symptoms of the mosaic although they were allowed to feed on the wheat until the plants died. The writer noted that plants affected with dry-land foot rot also had mosaic symptoms and extracts of these plants have uniformly produced mosaic in healthy potted plants. This suggests that a relationship exists between western wheat mosaic and dry-land foot rot in early planted wheat in Colorado.

Studies on the perithecial stage of Physalospora obtusa. AYCOCK, ROBERT. Perithecia of *Physalospora obtusa* occur abundantly in North Carolina on dead apple branches in the tree and on the ground. Periods of heavy ascospore discharge precede primary infections of apple leaves in the spring. These facts and other observations suggest that the sexual stage has a more important etiological role than is generally recognized. Cytological studies have revealed an unusual type of ascospore maturation. Following the third nuclear division in the young ascus 8 uninucleate spores are delimited by a process of free-cell formation. These young ascospores, which are about 3 μ in diameter, elongate until they measure about 3 by 24 μ. In unstained preparations they appear as long, filiform, intertwining strands. Subsequently, a marked increase in width occurs. Mature ascospores are ellipsoidal and average 13 by 30 μ. The young ascospores remain uninucleate until they are about 3 by 18 μ, after which several nuclear divisions occur prior to maturity, when as many as 16–20 nuclei have been observed. Comparisons of 24 single-ascospore cultures from 3 asci showed no clear-cut cultural or morphological differences.

Effects of excess solutes upon damping-off in sand cultures. BEACH, W. S. Using quartz sand in 8-inch stone jars as a medium, tomato seedlings were grown in six different strengths of Knop's solution, $\frac{1}{2}$, 1, 2, 4, 8, and 16$\times$ normal. Measures were taken to restore water lost by evaporation from the jars and to maintain pure cultures. At the time of planting (200 seeds per jar) the inoculum of a damping-off fungus was introduced in a circular pattern. It was found that Knop's solution, when added in concentrations stronger than 2$\times$ normal, reduced the growth rates of both the seedlings and the fungi. There was more infection with *Rhizoctonia solani* and *Fusarium* as the 16$\times$ normal solution was approached, however there was more infection with *Pythium ultimum* in the weaker solutions. *P. ultimum* did not grow in the 16$\times$ normal solution, and seed inoculated with this fungus did not emerge at this concentration. Physiologic damping-off occurred with an abrupt rise of temperature and rapid concentration of the solutes.

Multiplication of clover club-leaf virus (Aureogenus clavifolium) in its insect vector. BLACK, L. M. A viruliferous female of *Agalliopsis novella* was mated with a non-viruliferous male. The pair was caged on a Grimm alfalfa plant (*Medicago sativa*)

because alfalfa is immune from the virus. The alfalfa was grown in soil that had been steamed to kill all weed seeds. Of the 42 progeny, 21 were tested individually on crimson clover (*Trifolium incarnatum*); 15 produced infections. Therefore, on the average, the virus in the original female had been diluted at least 1:30 among her progeny. The remaining 21 progeny were each placed on an alfalfa seedling and when they became adult, the females were mated to non-viruliferous males. The pair with the greatest number of progeny was chosen to continue the line. The experiment has been continued on alfalfa for 4 years through 15 generations without loss of infectivity. Non-viruliferous insects after feeding on the same alfalfa plants on which the 15 generations had been grown, failed to infect any of 275 clover plants, whereas, control insects inoculated 93 of 274 test plants after feeding on club-leaf clover. Assuming no multiplication, the estimated minimum dilution of the virus in the insects exceeded 10^{-17}. It is concluded that clover club-leaf virus multiplies in its vector and is both an animal and a plant virus.

Comparative studies of a virus disease of cucumber transmitted mechanically from sour cherry and several cucumber virus diseases. BOYLE, J. S., J. DUAIN MOORE, AND G. W. KEITT. A virus disease transmitted mechanically to cucumber from young leaves of sour cherry showing necrotic ring spot symptoms was studied comparatively with several cucumber virus diseases. Inoculations were made on the cotyledons of young, potted cucumber plants held in a greenhouse air temperature range of 20° to 28° C. Symptom expression, host range, and certain property studies indicated characteristic differences between the cherry virus and the cucumber viruses used. On plants inoculated with the virus from cherry, the cotyledons persisted as yellow, mottled, turgid, and functional organs. The apical growing point was killed and about 30 to 45 days after inoculation bud proliferation with elongation occurred in the axis of the killed growing point with the subsequent development of a compact rosette of many flowers and dwarfed leaves. No herbaceous host other than cucumber has been found. The cherry virus had a thermal inactivation point of 52° C. for 10 minutes and a dilution end point of 1–20. Expressed juice kept at room temperature was infectious for only 12 hours. Final conclusions regarding the identity of the virus (or viruses) from cherry inciting the cucumber disease have not been reached.

Umbrella disease of Rhus glabra and R. typhina caused by Botryosphaeria ribis. BRAGONIER, WENDELL H. A disease resulting in cortical necrosis, wilting, and eventual death of plants of *Rhus glabra* L. and *R. typhina* Torn. is caused by the current cane blight organism, *Botryosphaeria ribis* G. and D. The disease present in Iowa, Minnesota, Missouri, Nebraska, and Wisconsin is responsible for widespread damage to roadside and natural plantings. In culture the pathogen produces abundant pycnidiospores on a wide variety of media under temperatures of 25° C. to 30° C. only if exposed to sunlight or the light from incandescent or fluorescent lamps.

Some effects of Fermate and Bordeaux sprays on Concord grapes. BRAUN, ALVIN J. Concord vines sprayed two seasons with Bordeaux mixture, 4–4–100 and 8–8–100, and with Fermate (ferric dimethyl dithiocarbamate), 1–100 and 2–100, showed accumulative effects of the treatments. The data were obtained from control-pruned vines on which the number of buds was determined by the weight of the previous season's shoot growth. Three applications of Bordeaux mixture decreased vine growth as measured by length of shoot and petiole. Similar applications of Fermate increased vine growth. Berry size was decreased by Bordeaux mixture and increased by Fermate. Bordeaux mixture delayed ripening of the fruit. The vines sprayed with Fermate yielded more than the unsprayed vines (yields adjusted for disease control) whereas those sprayed with Bordeaux mixture yielded less. By taking advantage of the increased vine growth resulting from the Fermate applications, greater increases in yields may be expected. Early applications of Fermate decreased the retarding effects of later applications of Bordeaux mixture.

Studies on variation in leaf rust of wheat, Puccinia triticina. BROWN, A. M. AND T. JOHNSON. In experiments designed to throw light on causes of pathogenic changes that have recently taken place in leaf rust of wheat no evidence was found to support the idea that pathogenic variation might proceed, in the uredial stage, through nuclear interchange between races. In the study of the possible role of *Thalictrum* spp. in originating new rust strains, greenhouse infection tests with native American species produced aecia on *T. dasycarpum*, pycnia only on *T. dioicum* and *T. polygamum*, and no infection on *T. venulosum*. Out-of-doors infection tests produced no infection on *T. dasycarpum* and *T. dioicum* but produced numerous aecia on the introduced *T. glaucum*. Selfing studies in which *Thalictrum* spp. were infected with races 3 and 5 indicated that the latter was homozygous whereas the former was heterozygous, pro-

ducing races 3, 15, 32, 68, and three undescribed races. A selfing of race 76 produced cultures with bright yellow urediospores that were low in virulence (resembling race 1), a new race with deep yellow uredia, and races 1, 29, and 101 with uredia of normal color.

A canker of Chinese elm. CARTER, J. C. Branch and trunk cankers were observed on several trees of *Ulmus parvifolia* Jacq. in November, 1946, in a nine-year-old planting at Urbana, Illinois. Several fungi were found fruiting in the cankerous bark. Cultures of the fungus which was isolated most consistently from the cankers were used to make eight inoculations in June, 1947. The technique of inoculation was to grow the fungus on sterile oat kernels, insert two or three fungus-bearing kernels beneath a triangular flap of bark on a leader, and seal the wound with Parafilm. This fungus and reisolations of it obtained from cankers which developed from the 1947 inoculations were used to make ten inoculations in May, 1948. All eighteen inoculations were followed by the development of cankers. The cankers resulting from the 1947 inoculations measured approximately 3.5 inches in length; those following the 1948 inoculations ranged from 3.75 to 9.5 inches in length. Infection became apparent eight to ten days after inoculation. Developing fruiting bodies of the fungus could be seen in about twenty days. The fungus has been classified tentatively as a *Tubercularia*.

Transmission of viruses through cherry seeds. CATION, DONALD. The Mahaleb and Montmorency seed collected from trees apparently infected with the cherry yellows virus complex were germinated in February and grown four months in the greenhouse. Grafts from the Mahaleb seedlings to seedling peach indicated over 20 per cent disease transmission with half of the infected peach trees showing ring spot and the other half showing typical cherry yellows symptoms. Similar inoculations from Montmorency seedlings resulted in over 20 per cent disease transmission, but only the ring spot virus was transmitted through Montmorency seed. Noninoculated peach trees of the same lot served as controls and grew normally without symptoms.

The constituents of "341" as apple scab fungicides. CHANDLER, W. A., AND H. W. THURSTON, JR. In 1948, the mixed glyoxalidine made from commercial stearic acid was formulated as the acetate salt dissolved in isopropanol (Experimental Fungicide 341–C). Commercial stearic acid contains approximately 45 per cent stearic, 45 per cent palmitic, and 10 per cent oleic acids. To test the relative effectiveness of these constituents of commercial stearic acid, glyoxalidines were prepared from synthetic stearic, palmitic, and oleic acids. These were formulated as the acetate salts in isopropanol and coded 341–SC, 341–PC, and 341–OC respectively. The 341–OC was very injurious to apple foliage and fruit, and it poorly controlled apple scab (*Venturia inaequalis* (Cooke) Winter). At ¾ lb. active glyoxalidine per 100 gallons, 341–C, 341–SC, and 341–PC controlled apple scab on susceptible McIntosh variety better than standard Lime Sulfur-Flotation Sulfur. The 341–PC and 341–SC did not russet the fruit, and russet from 341–C was less than that from the standard schedule, confirming previous years' experience. The 341–C, 341–PC, and 341–SC in a lead arsenate, lime, nicotine insecticide schedule prevented build-up of European red mites so that special miticide sprays were unnecessary.

Mineral nutrition in relation to infection of the tomato by Septoria lycopersici. CHAPMAN, R. A. AND M. B. LINN. Tomato plants in quartz sand and solution cultures in the greenhouse were given purified nutrient solutions varying in the concentration of single nutrient elements. The plants were inoculated with *Septoria lycopersici* Speg. at the six-leaf stage. Data for comparison were based on the number of lesions per unit of arbitrary leaf area of individual plants, obtained by summing the squares of the lengths of the leaves. Significant increases in amount of infection were obtained when nitrogen was increased from 21 to 630 p.p.m. and when boron was increased from 0.05 to 1.5 p.p.m. Significant decreases were obtained when manganese was increased from 0.05 to 1.5 p.p.m. and when copper was increased from 0.002 to 0.06 p.p.m. No significant differences were obtained when potassium was varied from 23.5 to 705 p.p.m., phosphorus from 3.1 to 93 p.p.m., iron from 0.1 to 3.0 p.p.m., and zinc from 0.005 to 0.15 p.p.m. Deficiency symptoms were obtained with the lowest levels of nitrogen, phosphorus, potassium, manganese, boron, and iron, but not with zinc and copper.

Soil treatments with chemicals for the control of tobacco parasites. CLAYTON, E. E., J. G. GAINES, T. W. GRAHAM, AND F. A. TODD. In the flue-cured tobacco growing area chemical soil treatments are effectively controlling both weeds and diseases. Plant bed treatments with calcium cyanamide and urea are well established. The following newer materials offer promise: Nitrites, especially calcium and potassium, at 1 lb. per square yard as weedicides, nematicides, and fungicides; sodium azide, ¼ lb. per square yard as a nematicide and fungicide; allyl alcohol, 6 qt. per 100 square yards as a weedicide.

Combinations of cyanamide and sodium azide, and allyl alcohol and ethylene dibromide, gave effective weed and root knot control. In field experiments D-D (dichloropropylene and dichloropropane) and ethylene dibromide gave good commercial control of root knot and nematode root rot (*Pratylenchus* spp.). Heavy reinfestation occurred by the end of the current crop year. The fumigation treatments did not affect the incidence of sore shin (*Rhizoctonia*), but in one experiment D-D gave excellent control of stem rot (*Sclerotium rolfsii*). With severe nematode infestation up to 50 per cent increase in the yield of cured leaf was obtained. In some experiments leaf quality was not affected, in others it was lowered.

Antibiosis of Actinomyces strains to Pythium arrhenomanes, P. ultimum, and Rhizoctonia solani. COOPER, W. E. AND S. J. P. CHILTON. A total of 2452 cultures of *Actinomyces* from 42 soil samples collected in Louisiana were tested for antibiosis on Czapek's agar to *Pythium arrhenomanes, P. ultimum,* and *Rhizoctonia solani.* There were 734 or 30 per cent of the cultures antibiotic to one or more of the three species of fungi. Of these, 588 were antibiotic to *P. arrhenomanes,* 335 were antibiotic to *P. ultimum,* and 345 to *R. solani.* More cultures showed a higher antibiosis to *P. arrhenomanes* than to the other two species. Cultures of *Actinomyces* were found which were antibiotic to one of the above three fungi, to all three of them, and to any two of them. At least three and probably more antibiotic substances were produced by the *Actinomyces* cultures tested. The antibiosis of a culture to one species was no index of its antibiosis to the other two species. No correlation was observed between soil type and the occurrence of strains antibiotic to *P. ultimum* and *R. solani.* The results indicate that in an analysis of the antibiotic potential of the *Actinomyces* present in a soil the cultures must be tested against a specific fungus.

Further studies on stem anthracnose of Lima bean in North Carolina. COX, ROBERT S. *Colletotrichum truncatum* is pathogenic on species from 11 genera of the Leguminoseae, and it can overwinter on exposed Lima bean refuse. Its cardinal temperatures for growth in culture were 5°, 27°, and 35° C. On inoculated Lima bean leaves the spores germinate, produce appressoria from which infection hyphae penetrate the cuticle and form primary hyphae in and between epidermal cells, within 22 hours. Within 46 hours subepidermal cells are penetrated and contents collapsed. A vascular discoloration as far as 150 mm. from the point of inoculation is evident within 5 days after injecting spore suspensions into stems and petioles with a hypodermic needle. Demonstration of the fungus in the xylem elements in free hand sections and other evidence suggests that spores are transported in the xylem. In 1948 the average yields (bu.) per acre of marketable green pods from nontreated plots and from those sprayed with the naphthoquinone Phygon XL (0.5-, 1.0-, and 1.5-100), or with the dithiocarbamates Dithane Z-78 (1.5-100), Zerlate (1.5-100), and Fermate (3.0-100) were 191, 317, 297, 283, 355, 296, and 282, respectively; whereas, the percentages of spotted pods were 58, 57, 44, 33, 32, 58, and 55, respectively. Phygon XL was phytotoxic.

White tip of rice. CRALLEY, E. M. White tip, an important disease of rice in the United States, has heretofore been ascribed to mineral deficiencies in the soil. Recent investigations indicate that white tip is caused by a nematode. The symptoms of the disease vary considerably from those of a nematode disease caused by *Anguillulina angusta* (Butler) Goodey, described on rice in India but are very similar to a nematode disease, caused by *Aphelenchoides oryzae* Yokoo, observed by the writer on rice in Japan in 1947. The disease is seed-borne. Viable nematodes have been found between the glumes and berries of infected rice seed 8 months after the rice was harvested. The actual survival period of the nematodes in infected seed is not known. The disease has been successfully controlled in the greenhouse by treating infected Arkrose rice seed with hot water. Hot-water seed treatment at 52°–53° C. for 15 minutes reduced infection on the growing plants from 75 per cent to less than 1 per cent. Thus far, in the United States, losses from the disease in the field have been reduced by early planting (April) and by growing resistant varieties such as Fortuna, Nira, and Bluebonnet.

Three years' tests with various compounds for control of bacterial spot of peach. DAINES, ROBERT H. During the past three years more than 20 compounds have been field-tested against bacterial spot (*Xanthomonas pruni*). Of the materials tested, zinc sulfate-lime, 8–12 lb. per 100 gallons; Delmo–Z–lime (a basic zinc sulfate), 1½–6; copper–8 quinolinolate-lime, 1–12; Tenn. copper "26"–lime–1–12; and an experimental compound gave some protection. Of the better known materials that provided little or no protection are Sulfur–6; four of the dithiocarbamates—Zerlate, 1 and 2; Fermate, 1½; Manganese ethylene bisdithiocarbamate, 1½; and Dithane Z–78, 1½; the naphthoquinone Phygon, ½ and 1; and the glyoxylidine 341–B–1 gal. Although zinc sulfate-lime gave some protection against bacterial spot, severe foliage injury followed its

use in two of the last three years. During 1946, injury was confined to early spray applications. During 1948, however, foliage injury continued to develop during much of the season, and the crop on the zinc sulfate-lime sprayed trees was greatly reduced. Trees sprayed with several experimental compounds have consistently had more disease than nonsprayed trees. Where Gamtox was applied during the curculio period and water during the remainder of the season, as a coarse fog applied in a driving stream, bacterial spot was significantly increased as compared with the same treatment applied in a well-fogged spray at low pressure.

The phosphorus metabolism of three species of Helminthosporium as determined by use of radiophosphorus. DAVIDSON, R. S. The phosphorus metabolism of *Helminthosporium turcicum*, *H. maydis*, *H. carbonum* race I, and *H. carbonum* race II has been studied using radiophosphorus. Replicated single-spore cultures derived from isolates of the three species known to be pathogenic on corn were grown in a modified Czapek's nutrient solution, each culture having 0.025 mg. radiophosphorus initially available. The phosphorus content of the mycelial mats after ashing was determined by means of a Geiger-Muller counter. The average phosphorus content per mg. of ash after 90 days was as follows: *H. turcicum*, 2.07×10^{-6} gm.; *H. maydis*, 1.03×10^{-6} gm.; *H. carbonum* race I, 1.5×10^{-6} gm.; and *H. carbonum* race II, 0.97×10^{-6} gm. The average ash weight per culture for each species was 4.1, 6.4, 5.1, and 6.7 mg. respectively. One month following inoculation, individual spores of *H. turcicum* contained 3.0×10^{-11} gm. P, *H. maydis*, 3.4×10^{-11} gm., and *H. carbonum* race II, 0.7×10^{-11} gm. *H. turcicum* grown with 25, 10, 5, 2.5, 0.5, and 0.05 mg. of phosphorus per culture for 60 days utilized 4, 7, 12, 29, 88, and 86 per cent respectively, of the available phosphous.

Combating bean blight chemotherapeutically with benzoic acid and the salicylates. DIMOND, A. E. AND E. M. STODDARD. Systemic infections of *Xanthomonas phaseoli* on red kidney beans may be reduced significantly in incidence and severity through chemotherapeutic treatment with salicylates and benzoic acid. Greenhouse grown plants in sand are fertilized with modified Hoagland's solution at a nutritional level for maximum susceptibility. They are systemically infected by pricking the pathogen into a young node of trifoliate leaves. Chemotherapeutic treatment consists of watering the plant with an aqueous solution of the test compound at non-phytotoxic concentration. Under these conditions, salicylic acid is effective at moderate but phytotoxic at high temperatures, acetyl salicylic acid is moderately effective, and 8-quinolinol salicylate is erratic. Phenyl and methyl salicylates are ineffective. In limited tests with salicylic acid, timing of applications with respect to inoculation and total number of applications have been varied. Treatment is apparently most effective when applications are made after inoculation. As the number of applications after inoculation is increased, the amount of protection is increased. To a much smaller extent, increasing number of applications before inoculation also increases protection. These results imply that salicylic acid is readily mobile, but rapidly metabolised in the plant.

On the genetics of resistance to mildew in barley. FAVRET, EWALD A. Studies were made at the University of California, on the inheritance of resistance to *Erysiphe graminis hordei* physiologic race 3, in several barley crosses. It was found that Monte Criste and Engledow India varieties carry a dominant gene for immunity, which has been called Ml^m and it is linked with one of the Nigrate factors determined by Briggs. The resistance of West China barley is induced by a single recessive factor, which has been called ml^w. It is probable that the resistance of Gopal barley was governed by two major genes, linked with a crossover value of about 15–20 per cent. With the two factors above mentioned, nine genes are already known to be involved in the resistance to mildew race 3 in barley, the largest number of loci related with resistance to a plant disease.

Physiological and chemotherapeutic investigations of Ceratostomella ulmi. FELDMAN, A. W., NESTOR E. CAROSELLI, AND FRANK L. HOWARD. To standardize laboratory biotitration techniques and toxin production, mycelial weight, oxygen tension, age, pH, acid production, reducing substances, and polysaccharide yield were studied in liquid shake cultures of *Ceratostomella ulmi* and were measured against wilt indices of sand-grown tomato cuttings. Highest toxin titre was obtained with sodium citrate–HCl buffered shake cultures (pH 4.25) in 7 days. No correlation was found between polysaccharide production in liquid culture and wilting. Culture extracts produced 100 per cent wilt when polysaccharide formation was eliminated by buffering. Toxin adjusted above pH 7 with Na or $Ca(OH)_2$ was inactivated. Cultures grown under high oxygen tension produced no wilt. By being predisposed to the dark for 5 days prior to inoculation, all two-year-old seedlings of *Ulmus americana* were diseased and had typical Dutch Elm symptoms. Of the 104 chemicals screened in the laboratory, 15 possessed some chemo-

therapeutic properties and so were further tested in the field as sprays, direct trunk injections, and soil injections under pressure. Three chemicals suppressed the disease symptoms during the season. Their effectiveness was greatly increased by soil liming. Weekly carbohydrate anaylses of the check trees and 3 treatments indicated a correlation between carbohydrate reserve and symptom incidence.

Copper naphthenate dust and spray. FELIX, E. L. Studies on copper naphthenate preparations safe to plants were initiated because of: (1) the well-known effectiveness of copper naphthenate against a variety of fungi in mildewproofing of cotton fabric, (2) its ability to withstand leaching, and (3) the results of limited tests in the field of agriculture. Dust was prepared from copper naphthenate dissolved in Stoddard solvent by mixing the following parts by weight, drying, and pulverizing: 70 per cent copper naphthenate 50, Attaclay 65 (3.5 per cent Cu); 80 per cent copper naphthenate 45, Attaclay 64 (3.6 per cent Cu); 80 per cent copper naphthenate 50, Attaclay 60 (4 per cent Cu). A dust of 35 per cent copper naphthenate in Attaclay (3.5 per cent Cu) possesses excellent flowability and adherence. Concentrations higher than 40 per cent are not useful in dust. The maximum concentration of metallic copper in naphthenate emulsion which can be used safely on some plants appears to be 0.0625 per cent, equivalent to 1–1–50 Bordeaux or less. The following emulsion (parts by volume) gives excellent wetting and an extremely fine spray: copper naphthenate 80 per cent in Stoddard solvent (Cuprinol Division) 50, Emulphor AG oil soluble 20, Emulphor ELA 10, mixed, and water 3720. Stock emulsions also may be prepared. Copper naphthenate in emulsion seems to penetrate quickly into plant tissues, leaving no visible residue and consequently minimum exposure to weathering. The value of copper naphthenate dusts and sprays in fungicidal crop protection and chemotherapy is being studied.

Host nutrition in relation to development of Verticillium wilt of tomato. GALLEGLY, M. E. Bonny Best tomato plants were grown in constant drip sand cultures of 0.1, 0.5, 1, 2, and 3 times a basal salt solution and in culture with the basal solution low and high in nitrogen, phosphorus, and potassium. The experiments were conducted during winter months with the greenhouse air temperature held at 20° C. and with fluorescent lamps regulated to give 12-hour days. Inoculations were made after 30 to 40 days' growth by dipping the washed roots in a concentrated mycelium and spore suspension of *Verticillium alboatrum.* In varying concentrations of the balanced solution, disease development was least at the 0.1 level and increased with an increase in salt concentration. In the unbalanced solution low in nitrogen, disease development was decreased; in the other solutions, disease development was no different from that in the basal solution.

Host nutrition and predisposition in relation to development of bacterial wilt of tomato. GALLEGLY, M. E. Development of wilt (*Pseudomonas solanacearum*) in plants grown in sand culture at nutrient concentrations 0.1, 0.5, 1, 2, and 3 times basal during summer months was greatest at 0.1 and decreased with an increase in salt concentration; during late autumn and early spring months disease development was greatest at 0.5 and 1. The first results were reproduced under controlled 18-hour day length while the second results were reproduced at 12-hour day lengths. Variation in light intensity and sand culture temperature failed to alter the long-day disease curve. In sand cultures with nutrients low or high in nitrogen, phosphorus, and potassium, disease development during summer months was retarded in the high nitrogen solution and increased in the low potassium solution; during early spring months both unbalanced nitrogen solutions reduced disease development. Pre-inoculation treatments including soil temperature, air temperature, day length, light intensity, and soil moistures had little effect on disease development. Post-inoculation treatments showed maximum disease development to be favored by 36° C. soil temperature, 28° C. air temperature, short-day length, low light intensity, and medium and high soil moisture.

Inoculation experiments with Atlantic and Pacific Coast collections of Tranzschelia pruni-spinosae discolor. GOLDSWORTHY, M. C. AND JOHN C. DUNEGAN. Inoculation experiments with urediospores of *Tranzschelia pruni-spinosae discolor* from Fort Valley, Georgia and Yuba City, California were carried on in the greenhouses of the Plant Industry Station, Beltsville, Maryland during 1948. Leaf and twig infections were produced by both collections of the rust fungus on all 13 commercial peach varieties (5 from California and 8 from the Eastern Seaboard) tested. Leaves were susceptible as soon as they unfolded, with rust sori first appearing at the base of the blades. Bark infections however did not develop on the shoots until 6 to 8 leaves had formed, and the bark could still be infected 9 weeks after the shoot had started to develop. The Palora, a western variety, and Dixired and Dixigem, recently introduced eastern varieties, were the most susceptible of the 13 varieties tested in respect to bark infection, while Elberta

was the most resistant. These inoculation experiments indicate that the absence of bark lesions under natural conditions in eastern orchards, in contrast to their frequent occurrence in western orchards, is not due to a difference in the rust fungus in these two widely separted regions.

Influence of climate on incidence of Fusarium rot and dry rot in gladiolus corms. GOULD, CHARLES J. Fusarium rot (*Fusarium oxysporum* f. *gladioli*), the most troublesome disease of gladiolus corms in eastern and southern United States, is also severe in eastern Washington, but uncommon in western Washington except on recently introduced stocks. Dry rot (*Sclerotinia gladioli*) is the most important corm disease in the latter area. To study the role that climate plays in the development of these two diseases, corms of the variety Picardy were obtained from a stock that was severely infected with both diseases. Apparently healthy unhusked corms were selected from this stock and grown in the comparatively cool climate of western Washington at Puyallup, and in the warmer climate of central Washington at Sunnyside. At harvest, 76 per cent of the non-treated western Washington-grown corms had dry rot and 5 per cent had *Fusarium* rot. Comparable figures for the eastern Washington-grown corms were 17 per cent and 32 per cent, respectively. Temperature is believed to be the primary factor responsible for these differences. Average temperatures from May through September were 60° F. at Puyallup and 67° F. at Sunnyside. These results and previous observations indicate that rotation between different climatic areas might be a useful supplement to standard measures for controlling dry rot and fusarium rot in commercial gladiolus corms.

Phytotoxicity of certain fungicide treatments to narcissus bulbs. GOULD, C. J., W. D. MCCLELLAN, AND V. L. MILLER. Certain fungicide treatments applied to narcissus bulbs sometimes injure the bulbs and are followed by such distortion of the flowers that varietal identification is impossible. Bulbs treated with New Improved Ceresan (ethyl mercury phosphate), 2 per cent Ceresan (ethyl mercury chloride, or Ceresan M (ethyl mercury p-toluene sulfonanilide) for the control of basal rot (*Fusarium oxysporum* f. *narcissi*) have produced the following abnormalities: perianth segments were paler than normal, proportionately narrower, and smaller; trumpet was paler, smaller, often lacked the typical recurved edge (King Alfred variety), and was frequently constricted around the exserted stigma and anthers; blasted flowers represented the extreme degree of flower injury; affected leaves were yellowish green and sometimes slightly swollen in the upper portion; severely injured bulbs decayed from the center outward. Injury most often occurred when bulbs were treated within three days after digging or when kept moist for several days after treating. Injuries produced by Dowicide B (sodium trichlorophenate) or by the warm water-formaldehyde treatment for nematode control were distinctive. Flowers injured by Dowicide B failed to open normally and perianth segments were dwarfed and greenish-yellow. Bulbs treated at the wrong time with warm water and formaldehyde produced blasted or jagged distorted flowers and leaves mottled near the tips.

Nematode root rot of tobacco and other crops. GRAHAM, T. W. Nematode root rot caused by *Pratylenchus spp.*, is a typical brown root rot. It is a disease of economic importance throughout the Coastal Plain Region of southeastern United States. In South Carolina, with tobacco plants transplanted in early May, invasion of the roots begins a month later. Maximum nematode populations were found in the tobacco roots during late July and early August. By mid-August extensive root decay had occurred and coincidentally the number of nematodes in the roots rapidly declined. This decline was associated with a rapid build-up of nematodes in the roots of crab grass, a universal volunteer plant in tobacco fields during late summer and fall. Tobacco roots invaded by meadow nematodes break down and decay more rapidly than similarly invaded roots of corn, cotton, peanuts, or crab grass. Certain of these other plants, however, support larger nematode populations. In a field experiment set up to measure these differences, the comparative size of the nematode populations for equal weights of roots on August 5 was: tobacco, 1.0; cotton, 0.7; crab grass, 2.3; and corn, 18.7. Some of the grasses, and corn in particular, are preferred host plants for meadow nematodes. Tobacco grown after corn is often severely damaged by nematode root rot.

Inhibitory action of 2,4-Dichlorophenoxyacetic acid on Penicillium digitatum and Phomopsis citri. GUISCAFRÉ-ARRILLAGA, J. Among chemicals tested for prevention of post-harvest decay of oranges, 2,4-D showed strong inhibitory action against *Penicillium digitatum* and *Phomopsis citri*. Marked reduction in decay occurred in oranges dipped 3 to 5 minutes in 0.1 and 1.0 per cent solutions of two commercial preparations of sodium salt of 2,4-D (70 and 76 per cent acid equivalents). *P. digitatum* was the prevailing fungus. In other tests, conidial germination of *P. digitatum* and *P. citri* was

inhibited by the same dilutions of the two materials. A 75 per cent "micronized" 2,4-D acid was effective at 1: 3000 against *P. digitatum* conidia and 1: 10,000 against *P. citri* conidia. On Sabouraud's agar, the sodium salt (70 per cent acid equivalent) added at rates of 1: 300, 1: 500, and 1: 1000 strongly inhibited growth, *P. citri* being more sensitive than *P. digitatum*. Orange peelings, dipped in conidial suspensions of these fungi, then in 1: 100 to 1: 1000 solutions of the sodium salt, and placed in moist chambers, remained free of fungus growth for 10 days. Checks were covered with mold. Some inhibition occurred against a strain of *Penicillium notatum* from the University of North Carolina.

Cross protection tests between the Wisconsin pea streak virus and several strains of bean virus 2 from pea. HAGEDORN, D. J. AND J. C. WALKER. Several strains of bean virus 2 were secured from pea plants affected with mosaic which had been sampled from 23 Wisconsin fields in 1948. Of these, 14 yielded viruses which were infective on Idaho Refugee bean and conformed to the characteristics incited by bean virus 2. The virus appears to be the most widespread on pea in Wisconsin. Wisconsin pea streak was prevalent in the latter part of the season, occurring often in conjunction with mosaic. Cross protection inoculations were set up to determine any possible affiliation between strains of bean virus 2 isolated from pea and the virus inciting Wisconsin pea streak. The latter developed readily in peas infected with the former. When soybean was inoculated with the streak virus there was no cross protection against bean virus 2 indicated. The two viruses thus may act additively in reducing pea yields.

Sex in Ustilago Lorentziana. HANNA, W. F. AND E. M. HURST. The four sporidia derived from a single chlamydospore of *Ustilago Lorentziana*, the fungus causing smut of wild barley (*Hordeum jubatum*) were cultured separately. When the monosporidial cultures were grown together in pairs on non-nutrient agar, fusions occurred between the sporidia of certain pairs of cultures only. Seedlings of *H. jubatum* inoculated with a pair of monosporidial cultures whose sporidia were capable of fusing with one another produced smutted plants, whereas those inoculated with other pairs of cultures or with a single monosporidial culture remained apparently uninfected. It is concluded that *U. Lorentziana* is heterothallic and bisexual.

Post-harvest chemical dip treatment of peaches for control of brown-rot disease in the package. HEUBERGER, J. W., G. D. MUNGER AND P. L. POULOS. Various water soluble fungicides were tested. Peaches were brushed, graded, dipped for 30 seconds, drained 3–5 minutes, packed in standard open-staved bushel baskets with liner and lid, and stored at room temperature 3 days. In exploratory experiments on the Halehaven variety it was found that Hyamine 1622 (a quaternary ammonium compound), Mycoban (sodium propionate), and Dithane D-14 (disodium ethylene bisdithiocarbamate) did not effect control at non-injurious concentrations but that Isothan Q-15 and Liquid Lime Sulfur did. In a definitive experiment on the Elberta variety, percentage rot data were as follows: water dip, 78.4; no treatment (absolute check), 52.3; Isothan Q-15 (2 qt.–100) 33.3, (1 pt.–100) 37.6; and Liquid Lime Sulfur (4 qt.–100) 19.6, (2 qt.–100) 20.3, (1 qt.–100) 28.6. In this same experiment, percentage rot data when packed bushel baskets were dipped were: Isothan Q-15 (1 pt.–100) 35.6 and Liquid Lime Sulphur (2 qt.–100) 16.8. When *Rhizopus* was present, Isothan Q-15 (lauryl isoquinolinium bromide) increased the amount of rot, Liquid Lime Sulfur effected no control, and storage at 42° F. gave perfect control. Isothan Q-15 did not affect the appearance, odor, or taste of the peaches; Liquid Lime Sulfur did not affect the appearance or taste, but did leave a slight sulfur odor which was distinguishable only at close range.

Relation of time of cutting to rate of decay of beech, birch, and spruce under natural storage conditions. HILBORN, M. T. AND F. H. STEINMETZ. That the time of cutting influences the rate of wood decay has been generally accepted for some time, but it has usually been stated that after 1 year's storage there is no difference in durability regardless of when the wood was cut. Data have been obtained for 3 species of wood cut at different times of the year and stored in the woods. Beech and white birch cut in March showed a higher rate of decay than when cut in July or September. White spruce cut in September showed more decay than when cut in March or July. These differences were apparent at the end of the first season, and continued to be expressed and intensified during the next 6 years. Removal of bark and splitting of larger bolts at the time of cutting considerably lessened the susceptibility to decay.

Location of bacteria in healthy potato tissue. HOLLIS, JOHN P. Isolations from potato seedpieces, stems, tubers and seed reveal the presence of a varied and mixed

bacterial flora in the vascular tissues of all parts except seed. Isolates were obtained most readily from seedpieces and stems. The prevalence of bacteria in the seedpieces was in proportion to the degree of vascularization of the tissue. Many different types of bacteria were obtained from seedpieces. Only 3 types occurred repeatedly, but 14 were obtained more than once. Accordingly these 14 were considered as reference types. Isolates from stelar regions of stems and tubers of the same potato lot and from various seed lots were compared with these. Five of the 14 types were found also in stems, 3 in tubers, and none in seed. Seven of the 14 types were obtained from plants growing in both soil and peat. These results furnish evidence of the presence and location of a mixed, heterogeneous bacterial flora in the vascular elements of healthy potato plants and support the hypothesis that bacteria enter the plant through wounds made by the emergence of secondary roots. The mode of entry remains to be proven.

Serial mutations of tobacco-mosaic virus. HOLMES, FRANCIS O. Mutants of tobacco-mosaic virus, derived from each other in series, disclosed two characters that were strictly correlated, though other observed characters mutated independently. Low infectivity was associated invariably with inability of virus to move from the site of infection in tobacco and increased infectivity with change to ability to move systemically. Two non-systemic strains were studied in detail. One proved relatively stable, giving rise to no green-mottling mutants and to few yellow-mottling or necrotizing mutants. The other was less stable, giving rise to many green-mottling, but no other, types of derivatives. Systemically-moving mutants were recovered without admixture of the parent strains, which had been used as original inocula, by subinoculation from newly developing leaves at the tops of the plants.

Bimodal dosage-response curves in fungicide field trials. HORSFALL, JAMES G., SAUL RICH, AND R. A. CHAPMAN. Fragments of bimodal dosage-response curves have resulted from field trials with several fungicides and several plant diseases. This is contrary to the expectation that effectiveness should decrease as dosage is decreased. Zinc ethylenebisdithiocarbamate (Z–78) sprayed on celery to control either *Septoria* or *Cercospora* yields a typical curve in which the protective action of the fungicide falls, rises, and falls again as the dosage decreases. The initial drop is due to the expected tendency of disease control to decrease with dosage. However, as Mason (1948) suggested, and as observed in our laboratory, as dosage decreases, a corresponding decrease takes place in the size of the aggregates of particles in the dried spray residue which expose a disproportionately greater specific surface area from which the toxicant can be dissolved. Increasing the specific surface area causes a higher rate of solution. The loss in toxicity due to decreasing dosage takes place more slowly than does the increase in toxicity due to increasing rate of solution. As both forces are concerned in the slope of the dosage-response curve, the rate of solution eventually dominates the downward trend due to loss in applied dosage, and the resultant is manifested as increasing toxicity. The final decline in toxicity is probably due to 1) further decline in dosage and 2) the effect described by Roller (1932) in which rate of solution falls off as decreasing particle size approaches the colloidal range.

Breeding for scab resistant apples. HOUGH, L. F., AND J. R. SHAY. In apple seedling orchards at Urbana, Illinois, and Lafayette, Indiana, 4664 scab-resistant apple seedlings have been established for fruiting tests. Of these, 871 are from crosses of 12 different small-fruited *Malus* species with Wolf River or Twenty Ounce, and 3793 are from crosses of scab-resistant selections with 2-inch fruits crossed with Jonathan, McIntosh, and Delicious. All progenies were grown from seeds in the greenhouse and scab-susceptible segregates were eliminated by artificial inoculations. Susceptible × *M. floribunda* gave a ratio of 1 resistant: 1 susceptible and *M. floribunda* selfed gave a ratio of 3: 1. This indicates that scab resistance in this clone is dominant and controlled principally by a single factor pair for which *M. floribunda* is heterozygous. In other species, the inheritance is more complex but segregation in crosses with commercial apple varieties is distinct and ratios seem consistent.

Trapping sporangia of Phytophthora infestans as an aid in forecasting the development of late blight. HYRE, R. A. A series of eight spore traps was established from Virginia to Rhode Island. The traps consisted of 1 × 3 inch vaselined slides held in a vertical position in a vane and changed daily. In a period of about two months, 13 sporangia were trapped, with some question as to the identity of five of them. Late blight always occurred within 12 miles of the traps prior to the time the sporangia were caught. These data indicate that the traps were of little practical value for forecasting

the early occurrence of late blight in 1948. Trapping studies were also made with sporangia of *Pseudoperonospora cubensis* (B. and C.) to find a more efficient arrangement of the slide. In a 5-mile wind a significantly greater number of sporangia were trapped when the slide was placed in a horizontal position or into the wind on a 45-degree incline, with a shield in both cases, than when placed in a vertical position without a shield. The relative number of spores trapped follows: vertical position in vane, 1; horizontal position, 4.6; inclined 45 degrees, 5.5; slide in vertical position at exit of funnel 18 inches in diameter at entrance, 2.7.

Virus particles in vascular contents. JOHNSON, JAMES. By means of artificial water pressure, the vascular contents of virus diseased plants were obtained for electron microscopy. Materials of 24 virus diseases have been examined in this manner. Rod-like particles were found associated with 9, and spherical bodies apparently with 2 viruses. Cubicle crystals, not definitely associated with any virus, were occasionally observed. With 12 diseases, abnormal particles were apparently not regularly associated with the virus. Ordinary, yellow, or a mild strain of tobacco mosaic yielded typical rod-like particles in several solanaceous genera, including *Nicotiana, Lycopersicon, Physalis, Solanum, Petunia,* and *Capsicum;* and, in the non-solanaceous genera, *Digitalis, Eryngium,* and *Plantago.* Similar rod-like particles were obtained from potato-mottle, potato ring-spot, tobacco-streak, tobacco ring-spot, a garden pea mosaic, and cucumber virus 4. The failure to find virus particles by this method cannot yet be regarded as significant unless accompanied by inoculation tests showing the virus present in reasonable concentration in the vascular contents. The particles of the tobacco mosaic virus have been found in the vascular contents as early as 36 hours after inoculation. The variation of the virus particles in the vascular contents suggests the value of such material for studies on the origin of the particles.

The number of strains of the tobacco mosaic virus. JOHNSON, JAMES. Numerous single-lesion isolations of tobacco mosaic virus from a highly susceptible host were continued serially through many transfers. Various treatments were applied with the purpose of inducing variation. The tests for variation were largely based on symptom expression and behavior on tobacco (var. Havana). The age of the plants inoculated, the rate and vigor of plant growth, the temperature, and other conditions vary symptom expression greatly. Variations from such causes, especially when associated with strain mixtures, are likely to be misinterpreted as strain variations or mutations. Strains of indicated purity, as a result of multiple single-lesion transfers, have remained remarkably constant in successive transfers except when exposed to high temperatures, which may induce virus alteration. Variants rarely survive or are conspicuous in nature on tobacco, either because of competition with the ordinary tobacco mosaic virus, or because of the less favorable conditions for development on older plant tissues. About 20 distinguishable strains have been isolated from field tobacco mosaic. Most of these are similar to strains previously described. The belief that strains of the tobacco mosaic virus exist in much larger numbers, as a consequence of frequent mutation, does not appear to be justified.

Soil fumigation for cigar-wrapper tobacco in Florida. KINCAID, RANDALL R., AND GAYLORD M. VOLK. Triplicate plots were fumigated on each of three dates (Sept. 29 and Dec. 3, 1947, and Feb. 5, 1948) with each of two fumigants, Dowfume W-40 (ethylene dibromide), 15 gallons per acre, and D–D (dichloropropylene and dichloropropane), 23 gallons per acre, for comparison with nontreated checks. Plots fumigated in September were sowed in oats 12 days later. The growth of the tobacco crop transplanted on March 25, 1948, appeared much better with certain of the treatments than with no treatment. Control of root knot was best with the earliest application of each fumigant; D–D was slightly more effective in this experiment than Dowfume W–40. Control of coarse root (nematode root rot) was not so good as control of root knot. Following fumigation, there was a prolonged retention of ammonia nitrogen in the soil, extending into April with Dowfume W–40, and into May with D–D. The effects of fumigation on the ammonia level in the soil and consequently on the plant are entirely separate and apart from the control of nematodes, and should be differentiated for a proper understanding of fumigation.

Pathogenicity of Sclerotinia trifoliorum to some forage legumes. KREITLOW, K. W. Monoascospore isolates from apothecia of *Sclerotinia trifoliorum* on Ladino and red clover yielded several cultural types that varied from strictly mycelial forms to those that produced numerous sclerotia. Pathogenicity of monoascospore isolates was established on Ladino and red clover as well as on other species of legumes. Differences in

pathogenicity of each of four monoascospore isolates was determined with 60–70 plants of Ladino clover. Most species of *Trifolium*, *Medicago*, and *Melilotus* tested were susceptible to a composite sample of pathogenic isolates; several species of *Trifolium*, however, were resistant.

Winter injury versus disease in Wisconsin poplar plantings. KUNTZ, J. E., AND A. J. RIKER. Hybrid poplars have been reported to fail in the North Central Region primarily because of winter injury. However, the need in Wisconsin for such rapidly growing trees called for further experiments on selected sites. Thus, replicated plantings of cuttings from 33 hybrid poplars from the Northeastern Forest Experiment Station and from 6 local selections have been made at different times during the past seven years in eight widely scattered areas. Winter injury was encountered occasionally (1) with first season trees, (2) on the tips of succulent shoots, (3) in frost pockets, but was not a critical factor. Continued satisfactory growth was limited, however, by cankers that were usually associated with *Cytospora*. This was found fruiting abundantly and was consistently isolated from the most advanced margins of the cankers. *Septoria*, so important elsewhere, was observed and isolated only occasionally. Vigorous trees withstood the cankers best. These results indicate that the chief problem in Wisconsin is not one of winter injury but rather one of selection for disease resistance.

Reaction of potato varieties to the virulent ringspot virus. LADEBURG, R. C., AND R. H. LARSON. A single-lesion ringspot culture, isolated from a diseased Red Warba potato, was used as inoculum in testing 44 American and 4 British varieties, over a two-year period, in the greenhouse and in the field. Older American varieties, such as Cobbler, Triumph, Russet Rural, Green Mountain, Russet Burbank, White Rose, Early Ohio, etc., failed repeatedly to show symptoms when inoculated, while most of the more recently introduced varieties tested were susceptible as evidenced by a strong mottle or mottle-and-leaf necrosis. Tubers from inoculated hills were replanted for study of second generation symptoms. No diminution in severity of symptoms was noted in the second generation and most hills of susceptible varieties evidenced the disease. Field spread tests indicated approximately 25 per cent infection due to vine contact during the previous generation. Although the variety Ontario was rather susceptible, inoculation of about 150 individual-tuber clones showed that about 25 per cent of them were highly resistant. A similar situation held with several other new potato varieties. The basis for this resistance is considered to be due to the type of the latent virus originally present in the clone, before inoculation with the virulent ringspot virus.

Isolation of virulent ringspot virus from "healthy" potatoes. LARSON, R. H., AND R. C. LADEBURG. A virulent ringspot virus was isolated readily from the ordinary latent virus universally present in older American potato varieties by means of single lesion transfers from the mottle-ringspot complex as expressed on *Nicotiana tabacum* and *N. rustica*. Isolations were made from the following varieties: Green Mountain, Russet Rural, Russet Burbank, Mohawk, Triumph, Cobbler, Houma, Red Warba, Gold Coin, and Beauty of Hebron. Physical properties included: thermal inactivation, 70° to 72° C.; dilution end point, about 1 to 1,000,000; aging in vitro, over six months. These properties checked very closely with those of virulent ringspot occurring naturally in potatoes. The ringspot virus was manifested in the form of three distinct types of local lesions on *N. rustica*, being designated as clear ringspot, necrotic ringspot, and necrotic spot. Pure cultures of the three types were also obtained by means of single lesion transfers. When inoculations were made at 16° C. to Red Warba and Pontiac with each of seven cultures of ringspot derived from the ordinary potato latent virus complex and eleven cultures of virulent ringspot isolated directly from potato, there were considerable differences between individual isolates as to virulence but the ringspot isolates derived from the latent virus complex proved fully as pathogenic, as a group, as the cultures isolated directly from potato.

Use of soluble and wettable fungicides for seed treatment. LEACH, L. D. To eliminate the objectionable features of dust application other methods of treating seed such as liquid fixation of dusts, slurry and spray treatment, have come into use. The last two methods require the use of either wettable or soluble fungicides. Numerous comparisons have shown that with uniform coverage spray applications of fungicides provide protection equal or superior to that from dust treatments. Soluble fungicides are more satisfactory for spray application because they eliminate the problem of maintaining suspension and reduce nozzle clogging. With beet seed a 0.15 per cent solution of ethyl mercury phosphate applied at 4 per cent of the seed weight was as effective against Pythium damping-off as Ceresan M (ethyl mercury p-toluene sulfon-

anilide) or Phygon (a naphthoquinone) at their usual dosages. Upon European sugar beet seed heavily infected with *Phoma betae*, Phygon XL, Arasan (a thiuramdisulfide), and Cerasan M applied as dusts provided inadequate control whereas the use of ethyl mercury phosphate as a spray (0.3 per cent solution) or a 20-minute seed dip (40 p.p.m.) resulted in over 90 per cent healthy seedlings. The maximum moisture application without drying depends upon the absorptive power of the seed surface. Four per cent is satisfactory on beet seed, but not more than 0.5 per cent should be used on Lima beans.

Resistance to anthracnose in sorghum. LEBEAU, F. J. During the past two years several hundred importations of sorghum have been tested for reaction to *Colletotrichum graminicolum* leaf spot. Under conditions of natural infection and also artificial inoculation disease development ranged from 0 to 100 per cent defoliation at the ripe stage. The domestic sorghum varieties used as controls were usually 100 per cent defoliated. Importations from India were, with few exceptions, also highly susceptible. Numerous highly resistant individual collections were isolated from among the African collection, which represented the bulk of the importations. Only a flecking reaction was produced on the more resistant collections artificially inoculated in the seedling stage with a spore suspension forced between the young unexpanded leaves with a hypodermic needle and syringe. Under similar conditions severe leaf spotting and necrosis of the expanding leaves occurred in susceptible varieties. In crosses involving two resistant collections with susceptible domestic sorghum varieties the F_1 proved to be highly resistant, in this respect reacting as the resistant parent. Preliminary F_2 data indicate a 3:1 segregation for resistance and susceptibility.

The determination of antimycin on plant leaves. LEBEN, CURT. Antimycin, an antibiotic that has been studied as a protectant fungicide, may be assayed on plant leaves by means of an agar diffusion method. Plants are sprayed with an antimycin suspension, dried, and discs ½ inch in diameter are cut at random from the leaves. Spores of the assay organism, *Colletotrichum circinans*, are seeded in acidified (pH 3.8–4.2) potato-dextrose agar plates. Leaf discs are placed, sprayed side downward, on the agar surface; likewise, blotting paper discs containing different concentrations of a standard antimycin preparation are tested. Plates are incubated at 28° C. for 40–48 hours and inhibition zones around the discs measured. A curve for the standard is plotted (the diameter of the zone of inhibition is a straight line function of the log of antibiotic concentration) and the amount of antibiotic on the leaf discs calculated in terms of "leaf units" by interpolation. The acidic medium prevents the obliteration of inhibition zones by rapid growth of bacteria from leaf discs; only occasionally have fast-growing fungi from discs prevented measurements. The deposition and wash-off of antimycin have been studied by this method. Leaves of apple, elm, tomato, cowpeas, and *Lonicera tatarica* have been used.

Cytoplasmic inclusions associated with wound-tumor in Rumex acetosa. LITTAU, VIRGINIA C., AND L. M. BLACK. Cytoplasmic inclusions have been found in cells of root tumors of *Rumex acetosa* and several other species of plants susceptible to the wound-tumor virus, *Aureogenus magnivena*. They were usually located in the undifferentiated cells of the tumor, but were occasionally observed in the tumor tracheids. They resembled nucleoli with respect to size, shape and reaction to different cytological fixatives and stains. Inclusions of this type were rarely observed in non-tumorous areas of roots from which tumors developed, and very rarely in meristems of uninfected roots. When stained with Flemming's triple stain, the nucleoli and inclusions stained red with safranin, while the nuclei were violet. The inclusions and nucleoli were Feulgen-negative. When stained with Unna's pyronin-methyl green mixture adjusted to pH 7, the inclusions and nucleoli were red (pyronin) and the nuclei blue. Digestion with the enzyme ribonuclease has failed to alter the staining properties of both nucleoli and inclusions in tests carried out thus far. The evidence from staining reactions indicates that the inclusions are composed of essentially the same materials as the nucleoli.

Chemical methods for the determination of dithiocarbamates in spray residues. MACK, G. L. Two types of dithiocarbamate compounds have come into use as fungicides; the dimethyl compounds, of which the iron and zinc representatives are already well established, and the ethylene bis compounds, of which the sodium, zinc, and manganese compounds are available. The tenacity and stability of the dithiocarbamates in residues vary widely, so that performance is difficult to evaluate on the basis of disease control alone. Colorimetric methods for both iron and zinc dimethyldithiocarbamate have been developed based on their solubility in organic liquids. The ethylene

bisdithiocarbamates are distinguished by their insolubility in organic solvents, instability in acids, and strong adsorption from alkaline solutions. Print methods, which require no preliminary solution or concentrations, are therefore especially indicated. They were also applicable to dimethyldithiocarbamate complexes where distribution patterns are of particular interest. The dithiocarbamate residue is transferred in a letter press from the plant surface to a hardened filter paper moistened with sodium hydroxide. A stable, highly colored, brown copper complex is precipitated in the paper on immersion in a copper sulfate solution buffered to pH 5. The relative depth of color is a rough quantitative measure of the amount of dithiocarbamate present, and forms a permanent record of the determination.

Spray adjuvants to improve the sticking and distribution pattern of zinc dimethyldithiocarbamate on tomato foliage. MACK, G. L., AND W. T. SCHROEDER. The possibility of improving the adhesion and distribution pattern of zinc dimethyldithiocarbamate (Zerlate) on tomato foliage with various adjuvants was investigated by correlating disease control data with the evaluation of the fungicide deposits as determined by a chemical leaf print method. The following commercial preparations listed as stickers or spreaders were applied with Zerlate in 5-application schedules in five randomized blocks: Orthol K at 1 pt. and at 1 qt., Orthol D at 1 qt., B 1956 at 4 oz., DuPont Spray Adhesive at 1 pt., Colloidal Z1 at 1 lb., Veg-Oil at 1 pt., and Nufilm at 1 pt. per 100 gallons of spray. Prints were made from leaves selected at random from each of three positions on the plants immediately after spraying and again after weathering. A correlation existed between leaf deposit ratings and control of Alternaria defoliation, with one exception. B 1956 deviated from the relation: its control of disease was better than would be predicted from the leaf prints. Two materials gave poorer disease control than Zerlate alone. Redistribution of the fungicide from the upper to lower leaves was indicated. Different distribution patterns also were obtained with the various adjuvants. Orthol K, Orthol D, and Veg-Oil were the outstanding materials from the standpoint of deposit ratings and control of Alternaria defoliation.

The influence of temperature on the incubation of the wound-tumor virus in the insect Agallia constricta. MARAMOROSCH, KARL. Experiments were designed to find the influence of temperature on the incubation period of the wound-tumor virus (*Aureogenus magnivena*) in the insect vector *Agallia constricta*. They were carried out in chambers with controlled temperature and light. The temperature was kept constant day and night. The day-periods were 16 hours long, night periods were 8 hours. The light source consisted of 28 fluorescent tubes per chamber, giving about 560 metercandles. At 16° C. the minimum incubation period was 30 days. Thereafter, there were long and irregular intervals between transmissions (up to 22 days). At 26° C. the minimum incubation period was 14 days, with very regular transmission. No transmission occurred at 37° C. All checks remained healthy. Insects which completed their incubation period at 26° C. lost their transmitting ability almost completely at 35° C. and did not regain their transmitting power when returned to 26° C. When insects transmitting regularly at 26° were placed at 16° C., transmission became very poor and irregular, but returned to normal when the temperature was again raised to 26° C. A temperature of 0° C. almost stopped incubation, but upon return to 26° C. the incubation period was completed. The investigation demonstrates the importance of controlled temperatures in experiments with incubation periods in insects and shows the effect of temperature on both the incubation and transmission.

Strains of Phytophthora infestans capable of surviving high temperatures. MARTIN, W. J. Attempts to explain the unusual occurrence in recent years of late blight in the fall crop of Irish potatoes in Louisiana led to a study of the reaction of different isolates of *Phytophthora infestans* to high temperatures. Cultures of the different isolates on navy bean infusion agar were exposed at 36° C. for varying times. After exposure, the cultures were kept at 20° C. for several days after which transfers were made to determine whether they were still viable. Eight different isolates were used, including four from Louisiana, three from Minnesota, and one from Cornell. The maximum time survived by the isolates was as follows: The four Louisiana isolates, 6 days; the three Minnesota isolates, 4 days; and the Cornell isolate, less than 6 hours.

Factors influencing the sporulation of Colletotrichum lindemuthianum in culture. MATHUR, R. S. *Colletotrichum lindemuthianum* (gamma strain) does not readily sporulate on common synthetic media. A medium containing glucose, mineral salts, and neopeptone was most favorable for sporulation. When glucose was replaced with equivalent quantities of sucrose, xylose, and galactose, sporulation was equally good.

Mannose, maltose, fructose, and lactose were less favorable while sorbose, soluble starch, mannitol, and sorbitol were unfavorable. Neopeptone was by far the most favorable nitrogen source tested for sporulation. The following nitrogen sources, in order of effectiveness, allowed some sporulation: urea, glycine, arginine, asparagine, and sodium nitrate. The fungus was partially deficient for inositol and biotin. Sporulation was stimulated when small discs of vitamin starved mycelium were soaked in dilute solutions of inositol, biotin, a mixture of these vitamins with thiamin or in a water extract of spores and matrix. These results suggest that spore matrix is rich in the essential vitamins. Sporulation was best at 15° and 20° C., less abundant at 25° C., and rare at 30° C. There was no sporulation below pH 3.0. Sporulation increased suddenly at pH 3.6, was greatest between pH 5.2 and 6.5, and moderate up to pH 7.7. Light and aeration were without effect on sporulation.

Soil treatment with Arasan for the control of damping-off of certain vegetables. McKEEN, C. D. The effect of soil treatment with Arasan (50 per cent tetramethyl thiuramdisulphide) on the incidence of damping-off on some vegetables has been studied in greenhouse flats. Under conditions of temperature and moisture favorable to the trouble, as caused by *Pythium ultimum* Trow and *Rhizoctonia solani* Kühn, soil treatment with Arasan has proved to be very effective in combating this disease in peppers, tomatoes, spinach, and cucumber. The greatest protection against damping-off in these vegetables has been obtained from a combined soil and seed treatment with the fungicide. With use of both treatments, the pre-emergence phase of the disease has been reduced to a very low level and post-emergence damping-off has rarely exceeded 5 per cent up to 16 days after emergence.

Septoria avenae on oats in Iowa. MEEHAN, FRANCES, AND H. C. MURPHY. A leaf spot and culm infection developed on oats in Iowa during 1948. Early in April, a fungus identified as *Septoria avenae* Frank was isolated from elliptical chocolate-brown lesions (0.5 × 1 cm.) on leaves of Clinton seedlings. Necrotic areas enlarged and coalesced by late June and July, but dark brown oval centers usually remained perceptible, distinguishing these leaf spots from those caused by *Helminthosporium avenae* Eidam and *Colletotrichum graminicolum* (Cos.) Wils. Perithecia of the ascogenous stage, *Leptosphaeria avenaria* Weber were occasionally present in advanced lesions. Basal leaf infection typically spread to the adjoining sheath that became grayish-brown and speckled with pycnidial aggregates. Portions of the culm beneath these areas were browned and tended to break before harvest. Cultures of the fungus were grown in yeast extract medium, and mycelial suspensions sprayed on oat seedlings. Inoculations were successful in all 36 varieties tested. Single pycnidial isolates of *Septoria avenae* f. sp. *triticea* from barley and wheat developed the *Leptosphaeria* stage in culture. Perithecia were common on culms and leaves of infected wheat. Cultures of the wheat and barley form failed to infect oats and *vice versa*. There were indications of the occurrence of *S. avenae* throughout the northern and eastern states. Cultures were isolated from Ajax and Andrew oats from Pennsylvania and New Hampshire.

Cultural and parasitic races of Cercospora arachidicola and Cercospora personata. MILLER, LAWRENCE I. Cultural races of *Cercospora arachidicola* and *C. personata*, which differ in color, rate, and type of growth were obtained by isolation from infected peanut leaves from different geographical areas in the United States in 1946 and 1947. Monosporous lines from the various cultural races have remained very stable in culture. Inoculation studies in the greenhouse and field in 1948 with different peanut varieties, indicate that several of the cultural races of both species tested differ significantly in pathogenicity. Blackish leaf lesions with sharp margins characterize those caused by one biotype of *C. arachidicola* on Spanish variety No. 146, while light brown lesions with a small yellow border characterize those produced by another biotype of the same species on Spanish variety No. 146.

An indexing method for necrotic ring spot and yellows of sour cherry. MOORE, J. DUAIN, AND G. W. KEITT. Sour cherry trees free from necrotic ring spot and yellows have been obtained by a method that combines indirect and direct indexing on sour cherry. Dorman nursery stocks were potted and forced in the greenhouse after budwood had been cut from each tree and placed in cold storage. In the indirect procedure each tree indexed was inoculated at bud-break with buds from Montmorency known to carry necrotic ring spot. Expression of ring spot symptoms indicated the stock was ring spot free when inoculated; no symptoms indicated the stock had ring spot when inoculated. In the direct procedure scions from storage were used to inocu-

late potted sour cherry free from necrotic ring spot and yellows. Expression of ring spot symptoms on these trees indicated the tree being indexed was carrying necrotic ring spot; no symptoms indicated it was ring spot free. Ring spot symptoms usually occurred in one to three weeks at greenhouse temperatures of 20° to 24° C. Scions from all trees shown by both procedures to be ring spot free were taken from storage and budded to potted Mahaleb rootstocks that were breaking bud. Thus far all trees free from necrotic ring spot have been yellows free also.

The effect of wetting agents on brown rot control. MORGAN, OMAR D., AND DWIGHT POWELL. Copper 8-quinolinolate was selected from several chemicals by laboratory tests especially designed for this experiment as the best fungicide to use in field tests against *Sclerotinia fructicola* on peach. Three proprietary wetting agents, each at 300 p.p.m. and 20 per cent alcohol were used with this compound. After spraying the peaches, which were nearing the firm ripe stage of maturity, all trees were inoculated with a suspension of *Sclerotinia fructicola* spores. This inoculation was done after darkness with a power sprayer at 300 lb. pressure. Degrees of infection were recorded from field counts and from peaches brought into the laboratory. "Triton B 1956" was the most effective wetting agent with copper 8-quinolinolate and allowed 12 per cent brown rot. Copper 8-quinolinolate when used alone resulted in 28 per cent infection. "Santomerse S," "Sterox CD," and 20 per cent alcohol resulted in 19, 16, and 20 per cent infection, respectively. The check plot which received no chemical treatment had 38 per cent brown rot infection.

Leaf rust resistance within certain species and hybrids of Populus. NAGEL, C. M. Cotton-woods, which are an important tree species in shelterbelt plantings of the Plains States, frequently suffer a high mortality following planting. Although the causes of mortality are perhaps several, it appears that leaf rust, *Melampsora medusae* Thüm. is an important factor in survival. Leaf rust frequently causes complete defoliation anytime after midseason on *Populus deltoides* Marsh., the principle species grown in shelterbelts throughout the Plains States. The effects of extensive defoliation on carbohydrate reserves and its association with ultimate winter killing or hardiness is presently incompletely known. During the past several years approximately 250 selections consisting of clonal lines of *Populus deltoides*, *P. sargentii*, and hybrids involving numerous species of *Populus* have been obtained and tested for their reaction to cottonwood leaf rust. Certain of these clonal lines possess high resistance to leaf rust. The range in defoliation was from 97.7 to 6.5 per cent, indicating a wide range in their tolerance to this pathogen. Certain of these hybrids appeared to have immunity to the currently prevalent leaf rust fungus under field conditions.

Comparison of dust fungicides and formaldehyde in the control of onion smut. NELSON, RAY. Experiments from 1943 to 1947 comparing dust fungicides with formaldehyde revealed the superiority of the liquid treatments in the control of onion smut in heavily infested soil. Denser stands and satisfactory control of smut were obtained in moderately infested soil with Arasan at the rate of 1 lb. to 4 of seed mixed in the drill. Seeds pelleted with Arasan and other fungicides at rates of 1–4, 1–2, and 1–1 gave unsatisfactory control and caused injury manifest in thin stands. Other dust fungicides have been tested in pellets and in the drill mixed with the seed. With the exception of Dithane Z–78 (disodium ethylene bisdithiocarbamate), none has equalled Arasan (tetramethyl thiuram disulfide) in effectiveness and safety. Bridging in the drill limits the maximum application of Arasan mixed with the seed at 1 to 4. A drill was designed to dispense any desired amount of dust simultaneously with the seed. In 1948 with soil conditions exceptionally favorable for smut, Arasan and Dithane Z–78, diluted 1 to 3 with talc and dispensed at the rate of 2 lb. to 1 lb. of seed, gave superior control of smut and damping-off and increased stands 63 and 73 per cent, respectively, over those from the undiluted dusts applied 1 to 4.

Life history of Stemphylium causing red leaf spot of gladiolus. NELSON, RAY, AND MARIE MOOAR. *Stemphylium* sp. has been isolated from gladiolus leaves from Michigan, California, Florida, New York, and New Jersey. One New York isolate of September 16, 1946, on prune agar formed much larger stromata than other cultures and was subjected to varying environmental conditions, including freezing and drying. April 21, 1948, 19 months after seeding with *Stemphylium* sp. the plate contained *Pleospora* perithecia with mature asci. Single-spore cultures were obtained from ascospores expelled to the cover of the Petri plate. Typical *Stemphylium* red leaf spot resulted from infection of gladiolus leaves with these cultures. Re-isolates from the lesions formed much larger stromata than previous cultures. The perithecia are glo-

bose, irregularly clustered, and densely setose. The outer cells are thickened and black. Perithecia are 396–555 × 460–904, average 495 × 725 microns. The asci are cylindrical, thin-walled with a thick, gelatinous inner wall. Ascospores are 11–30 × 29–70, average 17 × 42 microns, and vary from light, transparent yellow-brown to dark red-brown. The number of cross and vertical ascospore walls varies greatly within one perithecium. Species determination has been deferred.

Potato virus disease research in Mexico. NIEDERHAUSER, JOHN S.

Inheritance of sex and pathogenicity in Ceratostomella fimbriata from sweet potatoes. OLSON, E. O. *Ceratostomella fimbriata* has been considered a homothallic species. Among ascospore isolates from self-fertile (perithecial) strains a number of self-sterile (non-perithecial) strains were obtained. Two of these were of particular interest as they formed perithecia where they grew together. One, named self-sterile 1, produced sclerotium-like bodies, functioned as a female strain, and was pathogenic to sweet potatoes. The other, named self-sterile 5, produced no sclerotium-like bodies, functioned as a male strain, and was not pathogenic to sweet potatoes. Ascospores from the perithecia formed by these two strains gave four strains in a 1: 1: 1: 1 ratio. They were the two parental strains, the self-fertile strain from which the two self-sterile strains were originally obtained, and another self-sterile strain which was neuter. Pathogenicity and non-pathogenicity were inherited in a 1: 1 ratio, the self-sterile 1 and self-fertile strains being pathogenic and the self-sterile 5 and new strain being non-pathogenic.

Relationship of Ceratostomella fimbriata from the Hevea rubber tree and sweet potato. OLSON, E. O., AND W. J. MARTIN. Self-fertile (perithecial) cultures of *Ceratostomella fimbriata* isolated from *Hevea brasiliensis* in Mexico and *Ipomoea batatas* from the United States were very similar morphologically but differed in pathogenicity. The isolates from *Hevea* were not pathogenic to sweet potato and the isolates from sweet potato were not pathogenic to *Hevea*. The two were alike in that they both gave rise to self-fertile and self-sterile strains among single ascospore cultures isolated from them. When a week-old culture of a self-sterile strain from sweet potato was spermatized with spores from a self-fertile culture from *Hevea*, perithecia formed within three days. Very poor germination occurred in the ascospores from these perithecia since only 2 of 300 ascospores isolated produced cultures. The two cultures were self-fertile. One was pathogenic to both *Hevea* and sweet potato while the other was pathogenic only to *Hevea*. Ascospores isolated from the two cultures gave self-fertile and self-sterile strains with the same pathogenicity as the parent. The evidence indicates that hybridization occurred between the sweet potato and *Hevea* forms of *C. fimbriata*.

Induced variations in two species of Botrytis causing neck rot of onion. OWEN, J. H. Treatment of spores from *Botrytis allii* with methyl-bis (B-chloroethyl) amine produced several mycelial variations similar to *Botrytis byssoidea*. These variations remained mycelial for 3 to 4 transfers before sporulation reoccurred. Similar treatments of spores from *B. byssoidea* resulted in mycelial variations of different growth rate. One of these variants when inoculated into onion bulbs developed spores much smaller than normal and nearer to the spore size of *B. allii*. A large variety of media containing onion extracts and many vegetable decoctions failed to induce sporulation in *B. byssoidea*, whereas *B. allii* sporulated readily on all media prepared. One isolate of *B. byssoidea* produced sclerotia on potato-dextrose agar at 4°–12° C. in 30 days; addition of 0.5 per cent uranium nitrate induced sclerotium formation at higher temperatures. The same treatment with uranium nitrate caused a reduction in the growth rate of *B. allii*, but had no effect on sclerotium formation.

Some histological relationships between Helminthosporium victoriae and a susceptible and a resistant oat. PADDOCK, W. C. The reactions of Vicland, a susceptible variety, and Clinton, a resistant variety, of oats, when inoculated with spores of *Helminthosporium victoriae*, were investigated by the use of cotton-blue stained, free-hand sections of seedling leaves which had been dusted with spores and placed in a moist chamber. Spores germinated by the formation of one or more terminal germ-tubes. One to three appressoria were usually formed at the terminals of the germ tubes. On Vicland, the primary hypha may pass intracellularly through the epidermis and thereafter, until the later stages, is mostly intercellular. Occasionally the primary hypha is bulbous and thick and is limited for some time to the epidermal cells. In cells adjacent to the hyphae, the chloroplasts are nearly always disintegrated. On Clinton oats the primary hypha is limited to the epidermis and is frequently no larger than the ap-

pressorium. When it is larger, it has a diffuse granulated appearance with poorly differentiated walls. Disintegration of the primary hypha begins some time after penetration so that 80 hours after infection it has a clear amorphous appearance much like cytoplasm of the suscept cell. No disorganized chloroplasts were found in Clinton.

The effects of Fermate on the yield of McIntosh apples. PALMITER, D. H. The continuous use of Fermate (70 per cent ferric dimethyldithiocarbamate) on McIntosh apple trees which received no nitrogen fertilizer over a period of six years increased the yield of fruit 57 per cent over that of corresponding trees that were sprayed with wettable sulfur. Annual soil applications of two or more pounds of N per tree increased the yield of sulphur sprayed trees over that of the unfertilized Fermate plots during the first two years of the test but in the last three years the Fermate plots have outyielded even the best nitrogen fertilized plots. In 1947 and 1948 the best sulfur sprayed and nitrogen fertilized plots averaged 9 boxes of fruit per tree per year compared with 17 boxes of fruit per tree per year from the Fermate plots. Several factors appear responsible for this increased yield where Fermate is used as the fungicide in place of sulfur. Fermate contains some nitrogen and in a spray season a tree may receive as much as ¾ pound of N. However, other factors such as reduced scab and spray injury to the foliage may be just as important. In 1948 most of the trees in the experiment had close to 100 per cent bloom, but blossoms and fruit counts indicated more than 50 per cent increase in fruit set on the Fermate sprayed trees.

Physiologic specialization and variation in Helminthosporium teres. PON, DICK S. *Helminthosporium teres* is composed of many cultural races that may be distinguished by the following characteristics: nature and amount of mycelial growth, color, zonation, rate of growth, size and shape of spores. Mutation in culture is not uncommon in certain races. The pathogenicity of certain races differs sharply on varieties of barley. The optimum temperature for growth of the organism in culture is 25° C., for infection about 20° C.

Peach brown rot: pre-harvest sprays and control in the orchard and in the package. POULOS, P. L. AND J. W. HEUBERGER. An experiment on the Halehaven variety was conducted to determine the relative effect of various fungicides on rot in the orchard and subsequent rotting in the package. Incidence of rot was estimated as 3 per cent when the first pre-harvest spray was applied. Four sprays were applied (July 10, 16, 22, 30); four rot counts were made (July 24, 28, 30, Aug. 3); and three harvests were made (July 28, July 31, Aug. 3). At each harvest, the fruit was brushed, graded, packed in open-staved bushel baskets, stored at room temperature (approximately 75° F.) for 3 days and rot counts made. Percentage rot in the orchard for the non-treated, Liquid Lime Sulfur (2 qt.-100), Liquid Lime Sulfur + B-1956 (2 qt.-1 oz.-100), Sulforon (a wettable sulfur) + B-1956 (4- 1 oz.-100), and Bioquin 1 + B-1956 (½-1 oz.-100) was 38, 34, 25, 23, and 14, respectively, while percentage rot in the basket was (average for the 3 harvest dates) 72, 54, 56, 45, and 38, respectively. Liquid lime Sulfur and Bioquin 1 were phytotoxic to the foliage. Bioquin 1 (copper-8-hydroxyquinolinolate) imparted a "rusty" color to the fruit. An experiment on the variety Elberta yielded similar results. The dithiocarbamates, Parzate, Dithane Z-78, and Zerlate, were relatively ineffective for control in the orchard and in the package. Various soluble dithiocarbamate fungicides failed to control rot in the orchard and were highly phytotoxic to foliage. A combination of Liquid Lime Sulfur + Sulforon (1 qt.-2-100) appeared promising.

Effect of air temperature on virus concentration and leaf morphology of mosaic-infected horseradish. POUND, GLENN S. A strain of turnip virus 1 inciting horseradish mosaic was found to occur in much greater concentration in horseradish grown at 16° C than at 28° C. This was also true for old leaves of rape plants infected with the horseradish virus, but in young leaves the concentration was higher at 28° C. When horseradish plants growing at different temperatures and showing a gradient of virus concentration were reversed in position, *i.e.*, plants moved from 16° to 28° and vice versa, a corresponding reversal in virus concentration occurred. At temperatures where virus concentration was low, symptoms were very mild or masked; where virus concentration was high they were severe and persistent. Temperature also produced a marked effect on leaf morphology. At 28°, the prevailing leaf type was broadly laminate; at 16° leaves were often reduced to extreme fern-leaf structures. At intermediate temperatures, intermediate leaf types were produced. A reversal in temperature resulted in a corresponding reversal in leaf morphology. There was no correlation between leaf type and virus concentration. Efforts to free horseradish roots and plants by treatments in hot water and hot air, respectively, were unsuccessful.

Watermelon mosaic in Wisconsin. POUND, GLENN S. For the past 3 years, watermelon and muskmelon plantings in a south central Wisconsin area have been affected with a virus disease. Ten per cent of watermelon plants were infected in some fields. Symptoms on watermelon consist of severe stunting, bud proliferation, irregular black necrotic lesions, and occasional mottling. On muskmelon, symptoms are very similar to those produced by cucumber mosaic viruses. On all varieties of pea tested, marked systemic mottling was followed by death. On bean, the virus produced characteristic local necrotic lesions and systemic top necrosis. Symptoms on Havana tobacco occurred as etch-like rings and ringspots or as an oak leaf pattern on a conspicuously yellowed leaf. The virus was widely infectious to hosts tested. In physical property studies inactivation occurred at: dilution, 1–100,000; ageing in vitro, 5 days; thermal inactivation, 70° C. Cross immunity tests indicated no relationship with either cucumber virus 1 or tobacco virus 1. In certain symptoms, properties, and host range, the virus appears similar to the tobacco ringspot virus.

Fruit tree disease control with a new type of spray-duster and mist sprayer. PRATT, ROBERT M., L. M. MASSEY, AND K. G. PARKER. A sprayer for fruit trees, developed at Cornell University, applies either wetted dusts or mist spray formulations. The spray, or spray-dust, is carried by an air stream delivered from a 3-inch slot 8 feet long set at an angle of 35° from the horizontal. The air delivery of the machine now under test is approximately 20,000 c.f.m. at 105 m.p.h. Means are provided to adjust the angle of the air streams to compensate for wind interference. There is a saving of water of approximately seven-eighths of the amount used in conventional dilute spraying. Control of apple scab, cherry leaf-spot, and peach leaf curl has been equal or superior to that obtained by conventional methods. Sulfur, lime sulfur, low soluble copper compounds, ferric dimethyl dithiocarbamate, 2-heptadecyl glioxalidine, dinitro ortho cresol, and insecticides and adjuvants have been applied. Experiments on the control of fruit insects have been made in cooperation with entomologists. Both the spray-dust and mist spray methods are effective, but the latter is more economical of materials and is mechanically simpler. Disease control and chemical deposit data indicate that adequate coverage is obtained with this machine, which offers advantages over conventional sprayers in ease, speed, and economy of operation.

Fungicidal activity of dinitrocaprylphenyl crotonate. RICH, SAUL, AND JAMES G. HORSFALL. 2,4-Dinitro-6-caprylphenyl crotonate (code name Cr 1639) is an effective acaricide which gave good control of potato late blight in the 1945 insecticide field trials of Dr. F. B. Maughan. Used at 2 lb. per 100 gallons, during the 1946, 1947, and 1948 seasons Cr 1639 gave fair control of bean anthracnose, of *Septoria* and *Cercospora* blights of celery, and good control of early apple scab. It produced some leaf scorch on all test plants except celery. Laboratory and greenhouse tests showed that the tenacity and phytotoxicity of Cr 1639 are due to the dinitrocaprylphenyl portion of the molecule, while the fungitoxicity is due to the crotonic acid portion. In the laboratory, a deposit of Cr 1639 on glass is fungitoxic before drying but not after drying. Upon drying, Cr 1639 hydrolyzes and loses crotonic acid by volatilization. For this reason the usual laboratory screening techniques would miss this compound. Toxicity was reasonably maintained by drying the deposit on a strip of leaf epidermis. In the field, Cr 1639 probably acts mainly as a therapeutant, and as a protectant only so long as the leaf cuticle can retain the crotonic acid. Possibly crotonic acid can be used as a therapeutant in spray programs for apple scab and other diseases.

The western "X" virus a cause of "little cherry" in Utah. RICHARDS, B. L., L. M. HUTCHINS, and E. L. REEVES. Transmission studies during the past two years have established the fact that "little cherry", as it is recognized in Utah, is an expression of the Western "X" virus in the sour and sweet cherries on Mazzard root stock. When buds and fruit spurs from sweet and sour cherry trees with "little cherry" symptoms are grafted into peach, characteristic leaf symptoms of Western "X" disease of the peach result; when grafted into sweet and sour cherries on Mahaleb root stocks, typical wilt and decline symptoms are produced; and when grafted into chokecherry (*Prunus virginiana* and *P. demissa*), Western "X", red leaf, and "little cherry" fruit symptoms develop. These results with the "little cherry" inoculum duplicate in every detail results obtained in previous years with Western "X" virus inoculum from both the peach and the chokecherry. The transmissibility and general nature of the "little cherry" in Utah is further shown by the fact that buds and fruit spurs from diseased sweet or sour cherry trees grafted into sour cherry on Mazzard roots induced "little cherry" fruits, rosetted leaves, and proliferation and persistance of stipules.

Ozone as a stimulant for fungus sporulation. RICHARDS, M. C. Ozone is generally considered to be a fungicide and is used in meat storage plants to retard the development of fungi. It has been used experimentally in apple storages for similar reasons. Certain fungi which produced few or no spores in culture when exposed to ozone produced spores abundantly. Sporulation was increased with *Alternaria solani* and *Alternaria oleraceae* with no impairment of germination. A third species of *Alternaria*, which did not produce spores normally in culture, produced numerous spores when growing cultures were exposed to ozone. Ninety-six per cent of the spores germinated. The squash black rot pathogen, *Mycosphaerella citrullina*, produces few or no pycnidia nor spores on potato-dextrose agar. When cultures are exposed to ozone, masses of spores are produced, none of which will germinate. Ozone will stimulate fungi to sporulate. With certain fungi, however, ozone appears to be toxic to the spores.

The vacuum duster for applying fungicides and inoculum to plants. RICHARDS, M. C. AND DOUGLAS MURPHY. The application of dusts to bean plants to control bean rust appears to be a useful technique in screening fungicides. The vacuum duster which is in general use in the screening of insecticides has been found to give a uniform distribution of rust inoculum and fungicide to both the upper and lower surfaces of bean leaves. The cotyledon leaves of the variety Pinto were used in the tests. Fifty milligrams of the diluent containing 0.5-per cent uredospores were best for making the inoculations. With some of the more active mixtures 50 mg. of a one-per cent concentration gave complete control. The poorer mixtures required 50 mg. of a 4-per cent concentration to give equal control. The low percentage of the active ingredient in the dusts necessary to give complete control illustrates the importance of good coverage. By observing the number and distribution of the rust lesions it was possible to determine differences in the nature of the diluents with respect to ease of mixing and uniformity of the mixtures.

Selecting white pine for resistance to blister rust. RIKER, A. J., T. F. KOUBA, AND W. H. BRENER. In 1938 and 1939 selections were made in four Wisconsin areas of 163 cone-bearing white pine trees. They had been exposed about 15 years to blister rust among Ribes having from 10,000 to 65,000 feet of live stem per acre. Over 1,000 grafts from them on 2–2 pine seedlings and 10,000 seedlings were planted. They grew vigorously in a rust nursery also planted with many Ribes bushes. Furthermore, half the trees were inoculated individually, placed under muslin cages, and kept moist for over 60 hours. After such severe inoculation over 99 per cent of the control seedlings were killed by blister rust. Most of the parent trees (as grafts) had more or less resistance; about one-fourth possessed high resistance. Such resistance occurred in one or more of these ways: (1) infection negative or reduced in amount, (2) needle lesions reduced to small spots or flecks, (3) stem lesions corked out (the rust fungus apparently died), (4) lateral stem lesions failed to reach the main stem before the twig died, and (5) cankers failed to form aecia. Seedlings from these wind-pollinated selected trees, however, had little more resistance than commercial seedlings.

Oat parentage and procedures for combining resistance to crown rust, including race 45, and Helminthosporium blight. ROSEN, H. R. Since all known Victoria derivatives that are highly resistant to race 45 of *Puccinia coronata* are susceptible to Helminthosporium blight, and since Bond derivatives, with some notable exceptions, are susceptible to race 45, while resistant to blight, the problem of combining resistance to the two has been met: (1) By incorporating new genes into Victoria or Bond derivatives as by crossing with Mutica Ukraina or other varieties which possess resistance to one or both diseases; (2) by utilizing those Victoria derivatives which, while exhibiting an intermediate but apparently genetically fixed reaction to race 45, are largely resistant to blight as in selections from the crosses Boone × (Harry Culberson-Fulghum w.t.) and (Fulghum-Victoria) × (Bond-Iogold); (3) by using Bond derivatives that show combined resistance as occasionally found when a Fulghum type is used as one of the parents; and (4) by utilizing mixed populations of any one cross and not breeding for uniformity. As an example of the last, selections have been obtained from the variety Traveler (Victoria × Custis) in which approximately 70 per cent of the plants are resistant to race 45 and susceptible to blight, while the reverse is true for 30 per cent of the plants. Other selections present different proportions.

Factors affecting lesion formation on Physalis floridana inoculated with potato virus Y. ROSS, A. FRANK. A study was made of the factors influencing necrotic lesion formation on plants of *Physalis floridana* Rydb. inoculated with potato virus Y (*Marmor upsilon* H.). Diluted juice of diseased plants of *Nicotiana glutinosa* L. was used in all tests. Lesion formation was favored by partial shading and by tem-

peratures between 17° and 21° C. The use of carborundum increased lesion-count about eight-fold. Continued use of inoculating pads without renewal of inoculum resulted in a rapid progressive reduction in number of lesions. About six strokes per half-leaf was found to be optimal. There was a progressive reduction in lesion-count as the time between inoculation and rinsing of leaves was increased. Several buffers and water were compared as diluents. Although borate buffers caused severe injury to leaves unless removed by rinsing within a minute following inoculation, the use of 0.1 M borate pH 8 gave more lesions than did that of any other diluent. This was followed in order by 0.1 M glycylglycine pH 7 and 0.005 M phosphate pH 7. Dilution curves showed an increase in lesion-count up to a dilution of about 1–10. This was followed by a decrease approximately proportional to the decrease in inoculum.

Studies of "Mist Blower" fungicidal concentrates for row crops. ROWELL, J. B. AND F. L. HOWARD. The application of fungicidal concentrates to row crops at rates of 3–5 gallons per acre by low-volume applicators has been investigated to evaluate this principle for disease control. Field tests in 1947 of oil-soluble fungicides applied with a modified Potts-Spencer "Mist Blower" demonstrated the necessity of fine particle size and a wide distribution pattern for coverage and avoidance of injury. Laboratory studies demonstrated that 2–4 times LD 90 of oil-dissolved fungicides could be safely applied to excised tomato leaflets as 4 μ droplets. A Potts-Spencer skid-mounted air-blast machine attached to a Case VI tractor was employed in the current season's tests. An adjustable 4-row boom was equipped with 4 fish-tail air nozzles in front of which were mounted 4 Fitzhenry-Guptill VS-67 liquid nozzles that sprayed the concentrate into the air stream. The droplets varied considerably in size. Large droplets of the oil-fungicide solutions severely injured the foliage. Injury was eliminated by emulsifying the fungicide-oil solution in water. Two organic copper salts, Procop 110 and Puratized 111–5, satisfactorily controlled late blight of potatoes as compared to the checks; yields were 275, 266, and 227 bushels per acre respectively.

Comparison of volatile soil fungicides. SCHMITT, C. G. Of over 600 soil fungicides tested in soil in pots at 80° F., twelve of the more effective volatile fumigants were compared to determine approximate minimal lethal dosages in soil for vegetative mycelium of *Fusarium dianthi, F. oxysporum* var. *lycopersici, Sclerotium rolfsii, Scl. delphinii, Phoma terrestris, Verticillium albo atrum, Pythium de Baryanum,* and *Rhizoctonia solani.* Chloropicrin (Larvacide), the most effective of the group, killed all of the test fungi at 7.6 gallons per acre when sealed in and at 42.6 gal. per acre under a water seal. Minimal lethal dosages for other effective fumigants were 61 gal. for allyl bromide and 76 gal. for allyl and ethyl isothiocyanate; 380 gal. for formalin, Iscobrome 1 (15 per cent MeBr in xylene) and D-D (mixture of 1,3-dichloropropene 1 and dichloropropane), and 456 gal. for allyl chloride and methyl bromoacetate. The above dosages may not be adequate for kill if large sclerotia are present.

Relation of the European corn borer to stalk rots of corn. SCHNEIDER, C. L. AND J. J. CHRISTENSEN. Presence of the European corn borer (*Pyrausta nubilalis* Hübner) in Minnesota complicates the problem of developing varieties of corn resistant to stalk rots. In 1947 considerable stalk rot accompanied corn borer injury in inbred lines and varieties of corn that had been comparatively free from stalk rots before the borer spread into the State. Rot developed in 80 per cent of the internodes injured by the borer, while noninfested internodes in the same stalks were free from rot. The borer provides ways of entry for fungi; movements of larvae distribute fungi inside the plant; the frass within larval tunnels furnishes an excellent medium for rapid growth of fungi; and larval injuries weaken the stalk tissues and increase the likelihood of fungal attack. Larvae usually are infested both internally and externally with microorganisms, including many of those that cause stalk rot.

The control of blossom-end rot of tomato with emulsified hydrocarbon sprays. SCHROEDER, W. T. A greenhouse experiment conducted during the spring of 1948 indicated that an emulsified hydrocarbon preparation (Dowax 222) might be of practical value for the control of blossom-end rot of tomato in the field. Accordingly, 40-plant plots consisting of no treatment, Dowax 222 at 1.0, and Dowax 222 at 2.0 gallons per 100 of water were arranged in random order in each of four blocks. Sprays were applied at the rate of 200 gal. per acre on July 8 and 16. Blossom-end rot counts made during the harvest season showed 55 per cent control for the wax emulsion at the lower concentration and 70 per cent at the higher. Yields of 14.3, 16.0, and 17.5 tons per acre of number 1 equivalent fruit from plots with no treatment, Dowax 222 at 1.0, and Dowax 222 at 2.0 gal., respectively, were correlated significantly with the control of

blossom-end rot. No significant differences among treatments were obtained with respect to number of harvested fruit per plant, size of fruit, or percentage of culls exclusive of that occasioned by blossom-end rot, indicating that the increase in yield was due to the control of the disease.

Transmission of purple-top of potatoes. SELF, R. L. AND H. M. DARLING. During the summer of 1948 purple-top was transmitted by bud grafts from naturally-infected Katahdin, Chippewa, and Sebago potato varieties to 33 of 67 Sebago plants in a field having only a trace of the disease. Early symptoms of the disease appeared in 35 days and were followed in 10 days by severe plant wilt and production of flabby tubers. Plants having purple-top in general field plantings were placed in large pots in insect-proof cages. Approach grafts to these plants resulted in transmission of purple-top to 11 of 55 healthy Katahdin and Sebago plants and symptoms of aster yellows were obtained on 6 of 123 *Nicotiana rustica* approach grafts. In the greenhouse, aster yellows was transmitted by dodder grafts to 3 *N. rustica* plants from 1 purple-top Sebago. During 1947 and 1948 viruliferous leafhoppers (*Macrosteles divisus*) occasionally transmitted purple-top to potatoes; only 5 definite and 10 questionable plants of 238 inoculated showed symptoms. Transmission of purple-top resulted from insect inoculation of aster yellows to the *N. rustica* half in one of 13 *N. rustica*-Sebago approach grafts with the *N. rustica* showing aster yellows symptoms before the appearance of purple-top on the potato.

The effect of low temperature exposure on the development of peach brown rot in common storage. SHARVELLE, ERIC G. AND C. L. BURKHOLDER. During the past three seasons brown rot has caused serious losses to the Indiana commercial peach crop in storage. Common fungicides applied in the orchard have not adequately prevented these storage losses. Comparable samples of peaches were harvested in duplicate from several orchards in 1948. One sample was placed immediately in common storage at room temperature, and the other sample was exposed to a 24-hour cold storage treatment at 40° F. before being placed at room temperature. The incidence of brown rot development was recorded daily over a 6-day period following harvest. In samples obtained from commercially sprayed orchards, cold storage treatment greatly reduced the subsequent development of brown rot in common storage. Brown rot losses were reduced from 41.8 per cent four days after harvest to 0.0 per cent in one commercial sample. Similar effects of cold storage were obtained with fruit sprayed with Phygon (2,3-dichloro-1,4-naphthoquinone). This evidence is opposed to the view commonly held by growers and fruit handlers that brown rot development is accelerated when fruit is removed from cold storage to room temperatures.

Effect of copper and certain dithiocarbamate fungicides on the control of Verticillium spot and bubble of mushrooms. SINDEN, J. W. AND JOHN B. YODER. Bordeaux mixture, Copper A (copper oxychloride sulfate), Fermate (ferric dimethyl dithiocarbamate), Zerlate (zinc dimethyl dithiocarbamate), Parzate (zinc ethylene bisdithio-carbamate), and Dithane were sprayed on mushroom beds in varying concentrations and at different stages in the development of the mushroom crop. Bordeaux mixture and Copper A injured mushrooms on which they were sprayed, and neither was effective in control of either Verticillium spot or bubble. Fermate, Zerlate, and Parzate caused no injury to mushrooms on the beds in concentration of one lb. per 100 gallons of water. These materials reduced the incidence of bubble by 50 per cent and of spot by much more than this. Another notable effect was the suppression of surface growth of mycelium on the bed without reducing yields. This is desirable under conditions prevailing in a mine. Parzate proved the most effective and is being used commercially by making three applications of 0.5 lb. per 100 gal. sprayed in amounts of one qt. per 10 sq. ft. of bed. The first application is made soon after casing soil is applied, the second between first and second breaks of mushrooms, the final one between the second and third breaks.

Some specific characters of Erwinia atroseptica and Erwinia carotovora. SMITH, WILSON L. JR. Fifty-eight isolates of the *Erwinia* soft rot group were used in these studies. The majority were isolated in the last few years from various decaying vegetables and from blackleg of potatoes. The group was divided into two species *E. atroseptica* (van Hall) and *E. carotovora* (Jones) Holland. The two species reacted differently in culture, mainly on their utilization of certain carbon compounds in synthetic media. *E. carotovora* utilizes ethyl alcohol (5 per cent), dulcitol (1 per cent) and sodium salts of uric, hippuric, and malonic acid at 0.15 per cent concentration. *E. atroseptica* isolates do not utilize these carbon compounds. Both species grew well on

a medium containing 1 per cent maltose, but *E. atroseptica* produces stronger acid in this medium than *E. carotovora*. All isolates rot potato slices. Only isolates of *E. atroseptica* produce blackleg of potato plants. These cultures were also transmitted on an artificially infested knife used in cutting healthy seed pieces. When such seed were planted, some rotted, resulting in missing hills, and some produced plants affected with blackleg. *E. carotovora* isolates were not transmitted in this manner. It is concluded that the blackleg organism, *E. atroseptica* should be considered a distinct species from the soft rot organism, *E. carotovora*.

Selenophoma spot, a new wheat disease for North America. SPRAGUE, RODERICK. *Selenophoma donacis* var. *stomaticola* (Baueml.) Sprague and A. G. Johnson appeared suddenly in moderately severe form on wheat, an unreported host, in extensive areas in the Palouse country of Washington and Idaho. The fungus caused pinkish buff, irregularly elliptical leaf spots in March, 1948 and later appeared in more severe form in June as an ashy circular leaf spot with a darker, narrow margin. Artificial inoculation resulted in a few sterile spots on Federation wheat and Kentucky bluegrass but none on smooth brome, oats, barley, or Canada bluegrass. The fungus is tentatively called race 12, of variety *stomaticola*. Varietal resistance in wheat to this race, varied from the highly susceptible Rex and Orfed, to the highly resistant Kharkof, Comanche, and Hymar × Elgin 3 (F_4 Composite).

Recent changes in prevalence of physiologic races of Puccinia graminis tritici in South-Central Mexico. STAKMAN, E. C., J. G. HARRAR, W. Q. LOEGERING, AND N. E. BORLAUG. It appears that races 17 and 56 of *Puccinia graminis tritici*, among the most prevalent races in the United States and Northern Mexico since 1939 and 1934, respectively, have now become firmly and widely established in South-Central Mexico also, where they were found only sporadically or not at all prior to 1944. Together they comprised 5, 13, 15, 28, and 34 per cent of the total isolates obtained annually in South-Central Mexico from 1944 to 1948, inclusive. Prior to 1945 races other than 17 and 56, almost exclusively races 19, 38, and 59, made up 93 per cent or more of the total isolates each year. From 1945 to 1948, however, the percentages were 87, 85, 72, and 66. In 1948 races 17 and 56 comprised 25 and 10 per cent, respectively, of all isolates; and both were widely distributed, although apparently most prevalent in and near the State of Guanajuato, where stem rust was destructively epidemic. There are important implications for the program of developing stem rust resistant varieties, as certain varieties hitherto resistant in South-Central Mexico will henceforth be susceptible if races 17 and 56 persist.

Electron microscope evidence on the mechanism of multiplication of tomato bushy-stunt virus. STEERE, RUSSELL L. AND ROBLEY C. WILLIAMS. Preparations of partially purified virus from *Datura meteloides* plants in the early stages of infection with tomato bushy-stunt are found to possess particles which suggest the mechanism of multiplication of this virus. These preparations are made using a relatively mild treatment which requires only 25 minutes from picking of the infected leaves to drying of the suspension on electron microscope screens. The procedure involves dilution, mild heating, adsorption of normal plant material onto Cellite, and centrifugation at 3,000 r.p.m. Preparations from month-old infections contain spherical particles of uniform size. Electron micrographs of preparations from young infections show occasional particles which vary from this normal uniform size, and range from those which are but slightly larger and have a noticeable rift down the middle to those which are distinctly double consisting of two full-sized particles.

Studies on penetration and infection of sugarcane stalks by Physalospora tucumanensis. STEIB, R. J. Previous results have indicated that the scales on the buds of sugarcane stalks become infected with the red rot organism (*Physalospora tucumanensis*) in a latent form. The organism has been cultured from buds which were surface sterilized in a bichloride of mercury solution for as long as 24 hours. Buds with infected scales develop red rot under favorable conditions. Penetration of the epidermis of bud scales by infection threads was observed 33 hr. after inoculation with conidial suspensions. A cap or overgrowth around the infection thread was found in both a resistant and a susceptible variety. Mycelium was observed in cells below the epidermis only where the epidermal cells were thin-walled. In the resistant variety studied, the cap was seen in epidermal and sub-epidermal cells. A gummy dark red material often filled the intercellular spaces below the point of infection. This material was present but in less amount than in the susceptible variety studied. The leaf sheaths of the young shoots of a susceptible variety became infected from these bud scales. Such infected buds often failed to germinate.

Malachite green suppresses symptom expression of tobacco mosaic virus in tomato. STODDARD, E. M. AND A. E. DIMOND. Bonny Best tomatoes grown in sand and fertilized with modified Hoagland's solution were watered with a solution of malachite green in water at a strength of 1: 4000. Applications were made on 10 successive days and on the day following last application, plants were inoculated with tobacco mosaic virus. One month after inoculation, only two of five plants showed slight symptoms of tobacco mosaic virus whereas 5 of 5 check plants had pronounced symptoms. Severity indexes of symptoms (maximum index: 100) were 95 for the check plants and 15 for plants watered with malachite green. Similarly striking differences have been obtained in repeated tests. These results confirm on growing plants those reported by Takahashi (Science 107: 226, 1948) in which tobacco mosaic virus was shown not to multiply in tobacco leaves in floating leaf culture when injected with malachite green.

Utilization of the Baermann method as a means of assay of root infection by meadow nematodes, Pratylenchus spp. TARJAN, A. C. In studies of the effect of selenium on meadow nematode infections in boxwood roots, it was found that an improved method for evaluating the effectiveness of therapeutical treatments was needed. A review of the literature yielded only the tedious method of dissecting out and counting the nematodes contained in the root tissues. Tests were therefore conducted to evaluate the usefulness of the Baermann apparatus in obtaining quantitative determinations of nematode populations in infected boxwood roots. Standardized weights of infected roots were immersed in Baermann funnels, and meadow nematodes were observed evacuating the roots for as long as nine weeks. Only 19.4 ± 1.1 per cent of the total number of nematodes evacuated the roots by the first day. Most satisfactory results were obtained by drawing off aliquots three times weekly for a period of three weeks when it was found that 95.4 ± 3.5 per cent of the total number of meadow nematodes had evacuated the roots. It was concluded that a three-week immersion of the roots in the Baermann funnels would furnish a usable assay of a therapeutic treatment in the case of the meadow nematode infecting boxwood roots.

A storage scald of apples caused by midsummer oil sprays. TAYLOR, C. F. The application of spray oils at ovicidal concentrations during July and August has resulted in an unusual type of storage scald of apples in West Virginia. This relationship was suspected in 1944–1945 and was confirmed by experiments in 1945 and 1946. The "oil scald" appears in storage as small, sharply delimited, circular hydrotic spots. They are centered around lenticels and are small, usually two or three millimeters in diameter or rarely as large as five millimeters in diameter. Larger lesions are the result of coalescence of smaller single layers of hypoderm which are plasmolyzed and discolored. The light brown color of the dead cells is modified by the color of the fruit: the color of the lesions varies widely dependent on whether the spots are on red, green, or yellow portions of the apple. The severity of the "oil scald" increases during the late storage period; but it is abundant long before normal breakdown of the fruit occurs.

A wilt-inducing polysaccharide from Fusarium solani f. eumartii. THOMAS, C. A. A wilt-inducing substance produced in liquid cultures of *Fusarium solani* f. *eumartii* was found to be non-dialyzable, not filterable through bacterial filters, soluble in water, and relatively insoluble in most organic solvents. The substance was precipitated from non-dialyzed culture filtrates by the addition of 2 volumes of ethyl alcohol. Precipitation with alcohol from dialyzed filtrates was effected only after concentration of the filtrates. The wilt-inducing substance was thermostable in neutral, aqueous solution and labile in strong acid solution. It was partially to completely inactivated by heat in non-dialyzed filtrates at pH 7.5 and in sterile Richard's solution at pH 7.5 but not in Richard's solution at pH 5.9. It appeared that the substance was removed with phosphates precipitated by heating the alkaline, nutrient solution. Several qualitative chemical tests indicated that the toxic substance was a polysaccharide. Hydrolysis of the polysaccharide resulted in loss of wilt-inducing activity. Amount of polysaccharide was correlated with wilt-inducing activity. Tomato cuttings placed in solutions of the toxic substance wilted primarily their stems. Wilted cuttings did not recover when placed in distilled water unless sections one-half inch or more in length were cut from the basal part of the stem.

Mutants of Agaricus campestris induced by uranium salts. WAHL, I. Two distinctly new mycelial types of *Agaricus campestris* appeared in a monosporous culture of the cream brown variety growing on potato-dextrose agar containing uranium nitrate, $UO_2(NO_3)_2 \cdot 6H_2O$, at the rate 0.1 to 1.0 gram per liter. One variant was white, fluffy, and grew rapidly, producing a mycelial mat with dry weight 5 to 7 times greater than

that of the controls on potato-dextrose agar plus $NaNO_3$. These characters remained constant in subcultures on potato-dextrose agar plus $NaNO_3$, except for several reversions to the original form. Spawn of this variant readily produced white fruiting bodies instead of the original cream-brown, and preliminary experiments indicate that it produces mushrooms earlier than the parental type, although more data are needed. Basidiospores of the white mushroom germinated readily and produced a fluffy mycelial culture. Colonies of the second variant induced by uranium nitrate are flat, pinkish, and distinctly zonate. This variant also yielded white fruiting bodies. Similar cultural mutants were induced by uranyl sulfate, $UO_2\ SO_4 \cdot 3H_2O$, and uranium acetate, $UO_2\ (C_2H_3O_2)_2 \cdot 2H_2O$.

Resistance in cucumber to scab. WALKER, J. C. Scab (*Cladosporium cucumerinum* (Ell. & Arth)) is the most destructive disease of cucumber in the important pickle-growing area of northeastern Wisconsin. Maine 2 variety has been highly resistant during the three seasons, 1946–1948. The optimum for disease development is about 17° C. and as the temperature increases above this the lesions tend to be corked off with little damage. Maine 2 maintained high resistance in the greenhouse under optimum conditions although infection was not precluded and watersoaked lesions appeared on the youngest leaves. Resistance was completely dominant in crosses with Chicago Pickling, National Pickling, and Stays Green, and segregation in the F_2 at the ratio of 3 resistant to 1 susceptible occurred when optimum temperature was maintained, while at higher temperatures a deficiency in the susceptible class was noted. Since Maine 2 is not a satisfactory type in the area mentioned, a program of back-crossing with standard pickle types is being pursued.

A device for planting root-rot organisms with maize seed. WERNHAM, C. C. AND R. E. PATTERSON. The device is a modification of the American standard hand drop planter. To model No. 61 a Sears Roebuck fertilizer attachment is added; model No. F 61 is rebuilt. Both models are redesigned to drop inoculum from the fertilizer attachment on top of the seed. Seed plus inoculum is planted 7–10 days early to invite a cool wet soil environment. Maize cultures consisted of 77 S_1 ears in 142 rows and 142 F_2 ears in 239 rows with bulk open pollinated corn every eleventh row serving as checks. In each row 57–60 kernels were planted. Results of 1948 are given on surviving plants in classes 0, 1–10, 11–20, etc. The open pollinated material had a higher survival value and a narrower class distribution. The root-rot organisms are cultured on cooked grain and are dried before using. One bushel of inoculum will plant 10,000 hills with the planter plate half open. The device appears to be applicable to root-rot inoculations with other large seeded plants e. g. beans, cucurbits, cotton.

Production of mutants in Glomerella as influenced by age of culture. WHEELER, H. E. AND S. J. P. CHILTON. Previous studies have shown that ascigerous isolates of *Glomerella* of the Plus A type produce a large number of minus mutants, while those of the Plus B type although similar in appearance produce few if any. A comparative study of the effect of age of cultures on the production of minus mutants by Plus A and Plus B strains was made with dilution cultures using ascospores. Plus A isolates from wild morning glory and soybean behaved similarly. Only a few minus mutants appeared when cultures 10–15 days old were used but they appeared in increasing numbers as the cultures aged up to 60 days when about half of the colonies were of the minus type. Plus B isolates produced no minus cultures until 30 days old, the number then increased gradually with age but remained very low. Mutations from Plus to Minus apparently are associated with changes at a locus "B". The frequency of mutation at this locus was approximately 1: 1000 from a 45-day-old culture of a nonperithecial conidial strain obtained from a Plus A isolate and 1: 10,000 from a similar conidial strain from a Plus B isolate.

Phytopathological observations in the Low Countries. WHEELER, WILLIS H.

The effect of various diluents on the infectivity of the potato X virus. WILKINSON, R. E. AND A. FRANK ROSS. *Gomphrena globosa* L. was used as a local-lesion test plant to measure the effect of various diluents on the infectivity of the potato X virus (*Annulus dubius* H.) in plant juice. Dilutions were made just prior to inoculation. More lesions were produced with inoculum diluted with distilled water than with that diluted with phosphate or borate buffers tested over a wide range of concentrations and pH values. Several amino acid buffers appeared to have some advantage over water for use as diluents. Inoculum diluted with 0.05 M histidine or lysine buffers at pH 7.5 produced about 36 per cent more lesions than did that diluted with water. Triethanolamine oleate, a wetting agent, at concentrations of about 0.06 per cent increased the infec-

tivity of the inoculum about 50 per cent over that of inoculum diluted with water. Higher concentrations of this and other wetting agents reduced the infectivity of the inoculum, especially when the plant tissue was frozen before the juice was extracted.

Electron micrographs of tobacco mosaic virus in crude expressed sap. WILLIAMS, ROBLEY C. AND RUSSELL L. STEERE. Electron micrographs of rod-like tobacco mosaic virus have usually been obtained from highly purified material, although some pictures have been published of the virus photographed in crude sap treated only by dilution with distilled water. It has recently been possible to photograph the virus (a wild strain grown in White Burley tobacco plants) in undiluted, untreated crude sap, which was allowed to dry as a thin film on the specimen screens of the electron microscope. The virus then appears in large bundles or plaques a few microns in length and a micron or so in breadth. The plaques are found to be larger as the age of the infection increases. Slight dilution or washing with distilled water of either the crude sap or the dried preparation on the microscope screen causes the bundles to break up into the characteristic individual rods heretofore photographed. The possible origin and identification of the bundles will be discussed.

Tomato anthracnose control in 1948. WILSON, J. D. AND H. A. RUNNELS. In an experiment on the influence of timing of spray applications on control, in which schedules were begun at 10-day intervals from June 10 to August 19 and ended 60 days later, it was found that beginning on July 10 or 20 was most effective. Fungicide comparisons (30 or more materials) again indicated that zinc dimethyl dithiocarbamate (Zerlate) still ranks at or near the top for anthracnose control. A test of eradicant sprays applied to the soil just before the plants fell over indicated that at least two of the materials tested may be expected to give some control of later fruit infection. The addition of various stickers, etc., to Zerlate showed little increase in control over the 2–100 formulation. A further comparison of numerous varieties, hybrids, etc., still failed to uncover any marked resistance to the disease, but considerable tolerance was exhibited by some lines. Another experiment, in which the tomato fruits were observed at 2- and 4-day intervals after picking, indicated considerable variation in breakdown after picking. Further tests with trellises indicated that keeping the fruits off the soil is more effective than any spray program in preventing infection.

Verticillium wilt of avocados. ZENTMYER, GEORGE A. A severe wilt and dieback of avocado trees (*Persea americana*) was identified during 1947–1948 as caused by *Verticillium albo-atrum* Rke. and Berth. The fungus was isolated from trees of the Fuerte variety (hybrid Mexican × Guatemalan), a budded Guatemalan variety (Anaheim), and Guatemalan seedlings, under conditions of natural infection. Affected trees were found on both Mexican and Guatemalan root stocks. Trees first show a slight wilting of the leaves, then leaves rapidly die, turn brown, and remain attached to the tree. Vascular discoloration is brown and is usually extensive from roots to affected terminal branches. Some dieback occurs, then new shoots usually appear following new xylem formation. Trees often "recover" completely, but may wilt again in subsequent years. Mexican and Guatemalan seedling avocados, inoculated by dipping the roots in a suspension of spores and mycelium, showed wilting and leaf necrosis in two weeks at soil temperatures of 15°, 20°, 25°, and 30° C., but not at 35° C. The isolate from avocado also infected tomato. Scattered diseased trees were found in all of the commercial avocado districts of California. Some of the affected groves were previously planted to tomatoes. The disease apparently was previously termed "collapse" or "asphyxiation" and attributed to excess moisture in the soil.

Soil fumigants for control of Phytophthora root rots. ZENTMYER, GEORGE A. AND L. J. KLOTZ. Several soil fumigants and fungicides were tested against two species of *Phytophthora: P. cinnamomi* causing root rot ("decline") of avocado trees, and *P. citrophthora*, cause of citrus brown rot and gummosis. The 1,3-dichloropropene, 1,2-dichloropropane complex (Dowfume N, Shell D-D) killed both fungi in inoculated soil in laboratory and greenhouse tests when injected at dosages of 50 gal./acre foot or over. 1,3-Dichloropropene is the toxic fraction; its toxicity to the fungi closely paralleled that of the complex. 1,2-Dichloropropane was ineffective as a fungicide. The normal soil microflora was not eradicated at dosages of N or D-D lethal to *Phytophthora*. In greenhouse and extensive field trials, injection of soil with Dowfume N or Shell D-D at 60 gal./acre or above has resulted in good control of avocado root rot when infested soil was treated, then replanted to avocados. Materials were applied in field trials with weed guns and tractor-drawn mechanical applicators (the latter in cooperative tests with Dow Chemical Co.). In laboratory and greenhouse tests, fumigating soil with

chloropicrin and methyl bromide and applying solutions of Dithane (disodium ethylene bisdithiocarbamate) have also killed the two fungi, although Dithane did not penetrate the soil adequately. Ethylene dibromide was fungicidal only at dosages of 100 gal./acre foot or over.

Symptoms and resistances of crop plants to charcoal rot and ashy stem blight. YOUNG, P. A. *Macrophomina phaseoli* (*Sclerotium bataticola*) causes gray to white bark on the stems of some legumes and other hosts. It causes rotting and hollowing of stems and tap roots, rotting of fruits, and wilting of leaves. Sclerotia color the diseased internal tissues gray or black. Pycnidia grow in the bark of legume stems. Degree of resistance is indicated by numbers: practically immune, 0; resistant, 1; moderately susceptible, 2; and very susceptible, 3. Resistance of various crops is as follows: *Crotalaria spectabilis* (late), 3; *C. spectabilis* (early), 2; *C. juncea*, 3; *C. mucronata* (Giant Striata), 1; *C. retusa*, 1; *Phaseolus vulgaris*, 3; *P. polystachys*, 0; *Vigna sinensis* (Iron), 1; *V. sinensis* (Purplehull), 3; Velvet bean and Mung bean, 2; Lima bean and Soybean (Ogden), 3; *Sesbania macrocarpa*, 3; Hubam sweet clover, 2; Guar, 0; Lespedeza, 3; cantaloupe, watermelon, and pumpkin, 3; sweet potato (stems), 1; cotton (upland), 0 to 2; okra, 2; tomato, 1; pepper (California Wonder), 2; pepper (Pimento), 0; potato, 2; asparagus, 1; turnip, 2; radish, 3; cabbage, 1; zinnia, 3; *Cosmos sulphureus*, 2; corn, 3; sorghum, 0 to 3; Sweet sudan grass, 1; German millet, 0; and Johnson grass, 0.

ANTHONY BERG
1888–1948

C. R. Orton

Anthony Berg, one of several children of Joseph and Barbara Berg, was born at Bloomer, Wisconsin, October 3, 1888. He received his early education in public schools at Bloomer, and entered the University of Wisconsin in 1908, from which he graduated with the Bachelor of Science degree in June, 1912. His interest in phytopathology developed under the late Professor L. R. Jones, and led him to remain another year for graduate work.

He completed the residence requirements for the Master's degree and accepted a position in March, 1913, as Assistant Plant Pathologist with Dr. N. J. Giddings at the West Virginia Agricultural Experiment Station, where he remained until his sudden death with a heart attack on February 15, 1948.

Mr. Berg completed the requirements and received the Master's degree from the University of Wisconsin in 1922. He spent the years 1922–24 at the University of Wisconsin in graduate work and completed all the requirements for the Ph.D. except the thesis. Returning to West Virginia University in 1924, he continued his duties there, and, in 1925, was made Associate Plant Pathologist in the Agricultural Experiment Station.

Berg's early work in West Virginia was divided between apple-cedar rust with Dr. N. J. Giddings and his own research subject, *Phytophthora infestans* on potato and tomato, through which he first became nationally recognized. In connection with the cedar rust investigations, he discovered a rust-resistant red cedar, *Juniperus virginiana*, which bears his name.

In 1929, Berg became interested in a disease of the apple tree commonly known as "measles," which was reported from the Kanawha Valley and other parts in West Virginia, as well as other States. His studies soon led to the conclusion that more than one specific disease was masquerading under the term "measles". He first isolated and described the fungus causing a new disease which he named "black pox" as distinguished from the Arkansas disease described by Hewitt, and still another confused but widely distributed bark disease of the apple which he described as "internal bark necrosis". This last subject engrossed his attention until his death, when he was preparing a comprehensive paper for this and related bark diseases of apple.

He was married January 25, 1937, to Genevieve Clulo, who was Assistant Professor in the University and Assistant Plant Pathologist in the Experiment Station. She had worked with Berg on the measles problem and, after marriage, continued her studies on the anatomical and physiological features of the disease. Fortunately, through her long interest, she is carrying on these studies and bringing the results to publication.

ANTHONY BERG
1888–1948

During these years, Mr. Berg spent much of his leisure time in collecting and breeding Azaleas and Rhododendrons. He brought together at Morgantown practically all the native and many of the introduced species of Azalea, a collection which is hardly matched in America. Plans are laid for the University to take over and maintain this valuable collection for scientific and educational use.

Garden clubs and nursery organizations sought Berg's services and knowledge on Azaleas and Rhododendron. He was a frequent speaker before these organizations. He was always most generous in giving advice when requested, but would never venture beyond his personal experiences which had proved to be sound.

Mr. Berg was a member of the American Phytopathological Society, the West Virginia Academy of Science, the American Association for the Advancement of Science, and Sigma Xi. He was survived by his wife, Genevieve Clulo Berg, and daughter Rita Ann, and a sister Kate and brother George, both from Bloomer, Wisconsin. His mother passed away April 15 at the age of 97 years, just two months after his death.

His colleagues have lost a loyal and valued friend who was always sympathetic and helpful in every way. "Tony", as his close friends called him, will be remembered always as a quiet, kindly person who was generous to a fault, and ever ready to help in any emergency. Phytopathology has lost, in Anthony Berg, a skillful and thoroughly trustworthy scientist who made several noteworthy contributions to science.

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AN ISOLATE OF PORIA XANTHA ON MEDIA CONTAINING COPPER

RAY R. HIRT

(Accepted for publication July 16, 1948)

Poria xantha (Fr. ex Lind) Cooke is an important wood decay fungus. An extensive survey of decay in wooden buildings in central New York State, conducted by the Department of Forest Botany and Pathology of The New York State College of Forestry, has shown this fungus to be associated frequently with decay of wood in houses and industrial buildings. *Poria xantha* also is one of two fungi that has been frequently isolated from decaying timbers taken from wooden boats in service on inland waters (2).

This fungus has been used also as a test fungus to evaluate the effectiveness of certain wood preservatives suggested for controlling decay in wooden boats (5).

Hartley (3) has pointed out that it is futile to draw general conclusions from tests with one fungus under one set of conditions. This may be due in part to the fact that some fungi react differently to different wood preservatives. For instance, *Poria xantha* is stated to be somewhat tolerant of sodium fluoride (1) and copper at low concentrations (5); and *Lentinus lepideus* Fr. tolerant to creosote and pentachlorophenol (4). It is necessary to be familiar with the different responses of wood decay fungi to wood preservatives if correct interpretations are to be made in evaluating the preservatives. In standardizing tests it is also important to recognize these different responses in order to select the proper test fungi.

Poria xantha has been grown in culture under various conditions at The New York State College of Forestry. While incubating the fungus on standard malt agar containing different concentrations of copper naphthenate, a peculiar pattern of cultural behavior was observed. The phenomenon was considered sufficiently important to be brought to the attention of those individuals working with wood decay fungi.

THE FUNGUS

The fungus used for this study was *Poria xantha,* Culture No. 213a of the Division of Forest Pathology, Beltsville, Maryland. It was isolated by Dr. Ross Davidson from decaying mahogany wood collected by the writer in 1942, from the transom planking of a fresh-water cabin cruiser. Sporophores and mycelium of the fungus were present on several of the transom planks and adjoining hull planks, but Cultures No. 213a and No. 213 were derived from the decaying wood.

In stock cultures the fungus was grown on standard malt extract agar. To observe the reaction of the fungus to copper in the nutrient agar, copper

naphthenate was added to malt agar in proper quantities to supply the desired amount of copper metal. Standard malt agar containing the following concentrations of copper metal on the basis of weight was prepared: 0.00001 per cent; 0.0001 per cent; 0.001 per cent; 0.01 per cent; and 0.1 per cent. Ten Petri dishes of each copper concentration were poured and also 10 controls without copper. A technique was devised which insured a uniform distribution of the copper metal throughout the agar. Plantings of the fungus taken from a single culture were made in each of the dishes. The cultures were incubated at approximately 28° C.

RESULTS

In all of the control cultures on malt agar as well as in all the dishes containing agar with 0.00001 per cent, 0.0001 per cent, and 0.001 per cent concentrations of copper metal the fungus grew in its characteristic manner, except for differences in rate. In agar with 0.01 per cent concentration of copper metal the fungus grew in only 2 dishes, and in only one dish that contained a concentration of 0.1 per cent copper. The original inoculum from each of the 17 Petri dishes in which the fungus had failed to grow was removed after 16 days and placed on fresh malt agar without copper. No growth occurred, hence it is evident that the mycelium had been killed in 17 of the 20 cultures containing the two highest concentrations of copper metal.

The daily average rates of diameter increase of the surviving cultures from the sixth to the tenth days after establishment on the culture media were: no copper, 0.95 cm.; 0.00001 per cent copper, 0.91 cm.; 0.0001 per cent copper, 0.80 cm.; 0.001 per cent copper, 0.77 cm.; 0.01 per cent copper, 0.40 cm.; and 0.1 per cent copper, 0.19 cm. The copper had an adverse effect upon the growth rate of the fungus.

Surviving cultures on malt agar containing 0.01 per cent copper metal. The visual appearance of the mycelium did not vary noticeably from that in the control cultures. Mycelia taken from the two cultures that grew on malt agar containing 0.01 per cent copper metal were subcultured on similar nutrient media where they continued to grow. However, plantings from these cultures never succeeded in becoming established on malt agar with 0.1 per cent copper. For convenience here this mycelium is designated as Culture No. 213–50.

Surviving culture on malt agar containing 0.1 per cent copper metal. In the young culture the mycelium formed approximately a solid circle on the agar. As the culture aged, the growth gradually assumed a less regular shape with several sectors growing relatively slowly. The faster growing area of the mycelium had developed numerous radiating strands of yellow compact hyphae. The visual appearance of the slow-growing and rapid-growing sectors of mycelium was sufficiently distinct to attract the attention of a close observer. Subcultures were made from these two different

sectors and hereafter are designated as: Culture No. 213–501, from the area with the faster growing mycelium containing strands; Culture No. 213–503, from the slower growing uniform mycelium between the areas with strands.

Culture No. 213–503 could be subcultured usually on malt agar containing 0.01 per cent copper. However, it could not be re-established on nutrient agar with 0.1 per cent copper metal, even though this culture was originally isolated from such a substratum. Cultures No. 213–50 and No. 213–503 seem to be alike in all respects.

Culture No. 213–501, when established, grows vigorously on malt agar containing 0.01 per cent copper and 0.1 per cent copper metal. However, if it is transferred to and grown upon malt agar without copper, only occa-

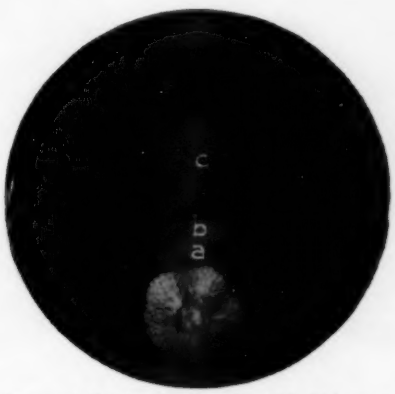

FIG. 1. A young culture of *Poria xantha* No. 213–501 on malt agar containing sufficient copper naphthenate to give a copper content of 0.1 per cent copper by weight. The mycelium is surrounded by two zones of faded agar: *a*, the darker zone; *b*, the lighter zone. The unaffected dark green agar is designated *c*.

sional subcultures are again successful on agar with 0.1 per cent copper. On agar with 0.01 per cent copper, growth is established more slowly when the inoculum is taken from malt agar without copper than with copper. Subcultures of the fungus direct from agar containing 0.1 per cent copper grow rather vigorously on malt agar containing either of the concentrations of copper metal, but more rapidly with the lower concentration (Fig. 2).

Malt agar containing 0.1 per cent copper metal is deep green. In successful cultures, approximately 48 hours after planting the mycelium of culture No. 213–501 on the agar, the green color of the agar begins to fade adjacent to the inoculum. This light colored region becomes wider and another narrow band of almost colorless agar appears outside the first faded area. The mycelium then begins to grow out from the inoculum (Fig. 1). After that, the cultures appear as a solid center of yellowish mycelium surrounded by two concentric bands of light colored agar enclosed within the deep green of the unaffected nutrient copper-agar. As

the mycelium grows the bands of light colored agar enlarge until they reach the walls of the Petri dishes. Apparently the faded bands of agar around the mycelium are fields of chemical activity associated with invasion by the fungus mycelium.

Solid cylinders of agar from the faded areas about the mycelium growing on 0.1 per cent copper agar were removed and placed on sterile 0.1 per cent copper agar. Mycelium from cultures No. 213a, No. 213–50, No. 213–501, and No. 213–503 were placed on the surface of these agar cylinders. Only the mycelium of culture No. 213–501 subcultured from agar with 0.01 and 0.1 per cent copper continued to grow. Apparently the loss

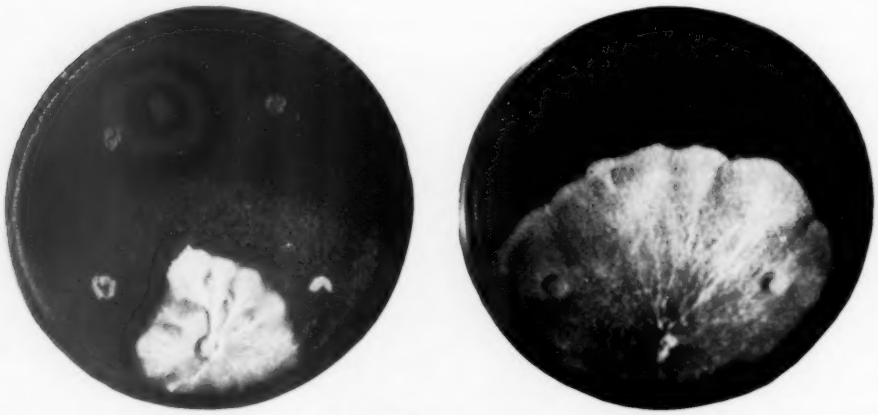

FIG. 2. Left: *Poria xantha* No. 213–501 growing on nutrient agar containing 0.1 per cent copper. Right: On agar containing 0.01 per cent copper. The unsuccessful plantings in each Petri dish are from the other cultures mentioned in the text. Cultures are the same age.

of the green color from the copper-agar due to the activity of mycelium No. 213–501, did not cause the agar to become a favorable substratum for the other 3 cultures.

As previously stated, on the agar with the high concentration of copper the fungus has a tendency to develop very compact rhizomorph-like strands of yellow mycelium (Fig. 2). It is from these strands that the most active growth occurs as the fungus spreads over the copper agar (Fig. 2, left). In subculturing the fungus onto new plates of nutrient agar with the highest concentrations of copper, more subcultures were successful if pieces of the strands were used rather than the more uniform mycelial growth between the strands. In fact, subcultures from the uniform growth areas were rarely successful on agar with 0.1 per cent copper, and it is possible that minute strands of the mycelium may have been present in those successful inocula. Mycelia from either area became established quickly on malt extract agar lacking copper.

It is possible that the parent culture of No. 213–501 represents a transfer of a portion of a rhizomorph-like strand from culture No. 213a. On

plain malt extract agar the compact strands are much less abundant than on agar containing copper. Therefore if the strands of compact hyphae are a factor in establishing the fungus on agar containing copper, fewer inocula from plain nutrient agar would be apt to succeed on copper-containing-agar than inocula taken from cultures on agar with copper, since in the former cultures the hyphal strands are relatively less abundant. The parent of culture No. 213–501 was the only successful culture from 10 plantings of mycelia No. 213a on 0.1 per cent copper agar.

Culture No. 213–503 was taken from slow-growing mycelium of uniform appearance that developed between the mycelial areas with rhizomorph-like strands growing on agar with 0.1 per cent concentration of copper. The slower growing mycelium lacks coarse strands and it also fails to form the tough skin-textured mycelium that can be peeled from the surface of the agar, such as that associated with the production of the coarse strands. This more uniform mycelium continues to grow between the strands but definitely slower than the strands themselves. This mycelium is associated with the strands and is probably composed of hyphae growing out from them, but it remains less compacted and may have a function other than that of directly extending the fungus on difficult substrata. Since culture No. 213–503 could not and No. 213–501 could be re-established on nutrient agar containing 0.1 per cent copper, and yet both grew vigorously on plain malt agar, apparently there is a fundamental difference between the two cultures No. 213–501 and No. 213–503.

Inocula from actively growing cultures of No. 213–50, No. 213–501, No. 213–503, and the original culture of *Poria xantha* No. 213a were placed at four separate points on malt agar in each of 5 Petri dishes. As the mycelial masses increased in size no differences were visible in the separate plantings, except for a somewhat more pronounced yellow in plantings No. 213–501. No lines of demarcation occurred between the separate mycelia, and eventually they grew together giving the appearance of a single culture in each Petri dish. This indicates, but does not prove, that *Poria xantha* No. 213a originally was the mycelium of a single fungus. The hyphae from the four cultures are similar microscopically.

SUMMARY AND CONCLUSIONS

Inocula from *Poria xantha* culture No. 213a were subcultured on nutrient agar containing copper in various concentrations. Copper adversely affected the development of the mycelium causing it to grow with more difficulty as copper was increased.

On agar containing 0.1 per cent copper metal only 1 planting of 10 succeeded in growing and this culture was characterized by rhizomorph-like strands. Portions of these strands when used as inocula readily established the fungus on other copper-containing agars with 0.1 per cent copper or less.

The mycelium between the strands was uniform in appearance and slow in growth. It could not be successfully transferred to and established on nutrient agar with 0.1 per cent copper, but readily grew on malt extract agar without copper.

Apparently the more favorable the agar substratum is for the growth of *Poria xantha* culture No. 213a, the less tendency there is to produce rhizomorph-like growths, and consequently the less chance there will be of successfully establishing the fungus on unfavorable substrata such as a 0.1-per-cent copper agar. On the other hand, if the fungus can be established on a nutrient agar containing as much as 0.1 per cent copper, the fungus produces many strands of compact hyphae which serve as favorable inocula for planting on similar copper-containing agars.

Apparently, such copper tolerance as *Poria xantha* may possess is associated largely with the ability of the fungus to produce strands of compact hyphae which, en masse, are able to endure until the fungus adjusts itself to the new environment. Even then, the fungus grows much more slowly than on nutrient agar lacking copper.

Unless care is exercised to secure uniform inocula from cultures of *Poria xantha* No. 213a growing on agar, it is possible to secure widely varying results in testing its reaction to copper-containing-substrata.

The results obtained in this study emphasize the statement already cited (3), that general conclusions regarding the effectiveness of a wood preservative may be very misleading when based on a single test or a single fungus. This is especially true where agar tests are used.

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SYRACUSE, NEW YORK.

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TRANSMISSION OF CHERRY YELLOWS VIRUS COMPLEX THROUGH SEEDS[1]

DONALD CATION[2]

(Accepted for publication August 4, 1948)

Certification of stone fruit varieties for the control of virus diseases is of interest to both fruit growers and nurserymen. In several States nurseries complying with prescribed standards of scion wood selection and a degree of isolation from possible disease sources are given certificates for freedom from disease so far as can be determined by inspection by the regulatory agencies. Such certificates are of limited value if seedling rootstocks are carrying viruses. Because many viruses may be masked in different species and varieties so that roguing is of nominal value only, an exact knowledge of the transmission of stone fruit viruses through seeds is of immediate importance.

Hildebrand[3] observed that certified budwood did not always result in disease-free trees since widely separated trees in the nursery row were affected with yellows. This was circumstantial evidence of rootstock contamination. He also observed abnormalities in occasional seedlings of *Prunus Mahaleb* grown in nurseries. An unspecified amount of indexing from such trees gave symptoms that he considered typical of ring spot and cherry yellows.

Cochran[4] detected visual symptoms of ringspot in seedlings that he grew from *Prunus avium* and demonstrated the transmission of virus through seed by transmitting it to peach from five of the six "Mazzard" seedlings tested but obtained no symptoms from six seedlings having no visual symptoms. His similarly grown Mahaleb seedlings from ring spot-infected trees showed no symptoms and apparently were inferred to be virus free as they were not reported indexed on peach.

METHODS USED TO DETERMINE SEED TRANSMISSION

The cherry yellows disease is considered a complex of at least two viruses, one of which gives symptoms identical to those of the ring spot virus while the other component has not been determined. Cherry yellows is not detectable in Mahaleb cherry by casual examination. It must be determined by indexing on reacting hosts such as sour cherry varieties or peach seedlings.

[1] Published with the approval of the Director as Journal Article 995 (n.s.) of the Michigan Agricultural Experiment Station.

[2] Research Associate in Plant Pathology, Department of Botany and Plant Pathology, Michigan State College, East Lansing, Michigan.

[3] Hildebrand, E. M. Viruses and cherry rootstocks. Amer. Nurseryman 82(7): 5–8, 18–21. 1945.

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To study the possibility of seed transmission of the cherry yellows virus components, seeds were collected in 1946 from Mahaleb sprouts growing from the roots of diseased Montmorency cherry trees. The seed was held out-of-doors in moist peat until October 1, then placed in cold storage at 40° F. for about three months. When individual seeds showed signs of sprouting they were planted in flats in the greenhouse. Only a few of approximately 1000 seedlings had any abnormal mottling or other possible symptoms. Similarly, seeds were saved and grown from a Montmorency tree considered to have typical cherry yellows symptoms.

Plant tissue from a random sampling of the normal-appearing seedlings was grafted in August, 1947, to yearling seedling peach trees growing in the field. Transmission readings were made during the first six weeks of growth in 1948, mostly during May.

Because of the limited number available, only 65 peach trees were used to index the seedlings from Mahaleb. Each peach tree received tissue grafts from three different Mahaleb seedlings. The percentage transmission thus could only be estimated. Likewise 79 peach trees were used to index 227 Montmorency seedlings.

EVIDENCE FOR SEED TRANSMISSION

The data in table 1 and in figure 1 illustrate that at least 42 of the 65 peach trees received one or more viruses resulting from inoculations with

TABLE 1.—*Transmission through seeds of Mahaleb and Montmorency cherry of component viruses concerned with the cherry yellows complex as determined by indexing on seedling peach trees*

Data recorded	Source of graft tissue	
	Mahaleb seedlings	Montmorency seedlings
Number of cherry seedlings indexed	195	227
Number of peach trees inoculated	65	79
Number of diseased peach trees:		
Necrotic ring spot[a]	20	68
Cherry yellows[b]	17	0
Indeterminate, trees killed[c]	5	2
Total	42	70
Number of peach trees with no reaction	23	9

[a] Delayed foliation, die back, later sprouts normal.
[b] Delayed foliation, die back, later sprouts rosetted.
[c] Delayed foliation and die back. Either ring spot or cherry yellows may have caused death.

tissue grafted from Mahaleb seedlings. However, as 195 Mahaleb seedlings were tested, the virus transmission was approximately 21.5 per cent. Of these, at least 20 or approximately 10 per cent transmitted ring spot virus while at least 17, or approximately 8.7 per cent transmitted the cherry yellows virus complex.

In the Montmorency determination, 79 peach trees received graft inoculations from 227 Montmorency seedlings. Of these, 9 peach trees remained normal while 68 showed symptoms typical of ring spot infection and two died from the result of virus infection. In case of death, the lack of sprouts made exact diagnosis impossible. The amount of disease transmission was at least 30 per cent. It is of interest to note that a greater number of cases of virus transmission through seed was demonstrated for Montmorency than for Mahaleb and that only the ring spot component of

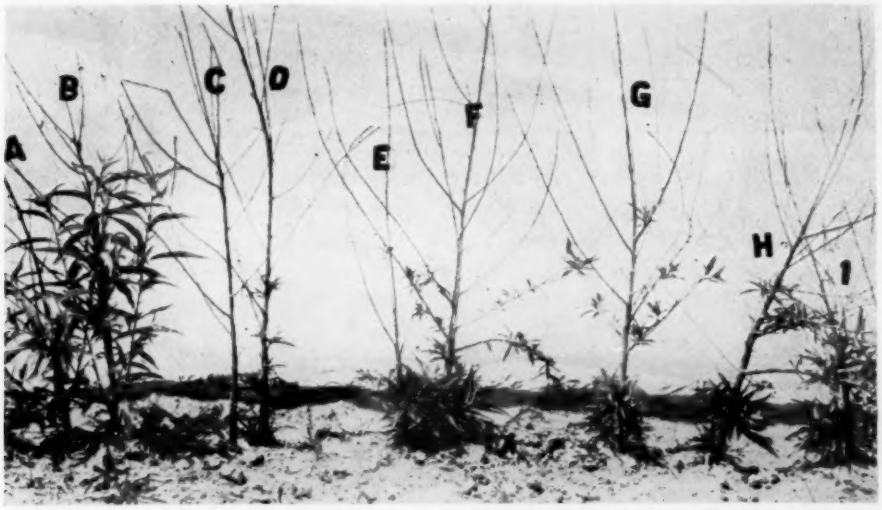

FIG. 1. Results of Mahaleb indexing on peach. Trees A and B show recovery from ring spot symptoms. Tree C is dead and trees D to I show the rosetted growth typical of cherry yellows on peach. Photographed June 2, 1948.

the cherry yellows complex was involved. Examples of peach reaction to Montmorency indexing are shown in figure 2,A and B.

The possibility that the Mahaleb and Montmorency seedlings acquired infection in the greenhouse is remote, because 75 previously disease-free Montmorency trees growing in the same greenhouse were similarly indexed and transmitted no infection to peach. The infection of seedling peach in the field from outside sources is improbable because none of the many control trees showed symptoms. Therefore, the data appear to offer conclusive proof of virus transmission through seed.

In another experiment, 325 cherry trees propagated from previously determined disease-free budwood and grown on a commercial source of Mahaleb stock were indexed and found to have 9 cases of cherry yellows and 5 cases of ring spot. In all cases only one of the several to many budlings propagated from an individual tree became diseased while all other budlings propagated from the same tree indexed disease free. The probable explanation is that some of the Mahaleb stocks were infected.

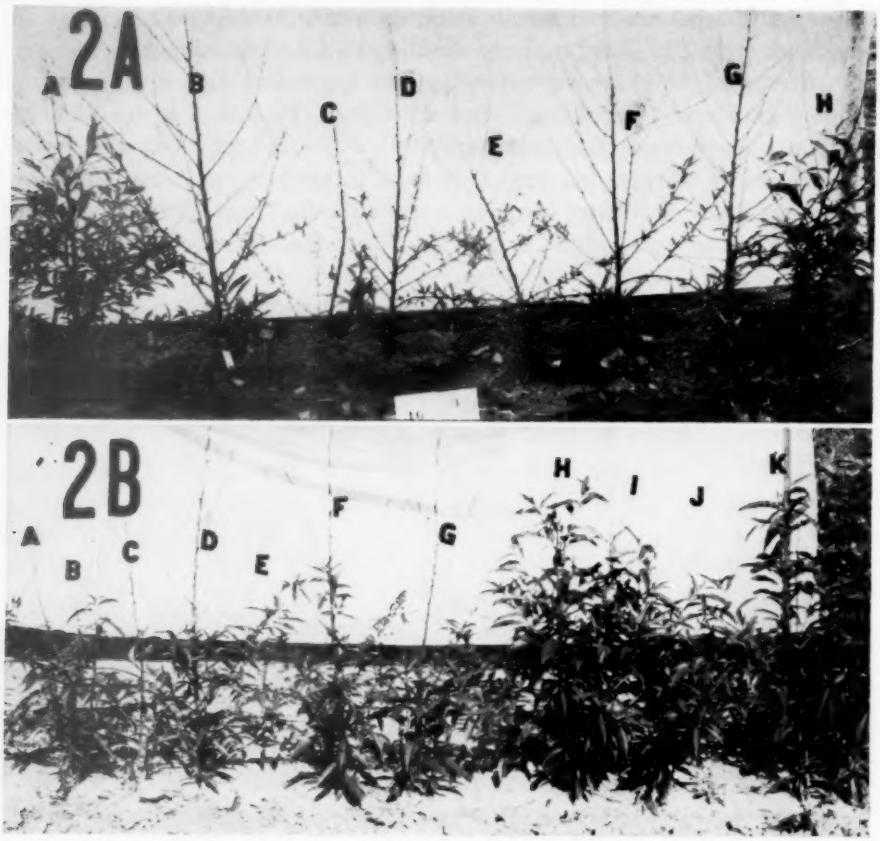

FIG. 2. Results of Montmorency seedling indexing on peach. A. Trees A and H are normal, while trees B to G show the delayed foliation typical of ring spot infection. Photographed May 15, 1948. B. Trees A to G show recovery following delayed foliation while trees H to K are normal noninoculated controls. Photographed June 4, 1948.

SUMMARY AND CONCLUSIONS

Mahaleb and Montmorency seeds collected from known cherry yellows-diseased trees were grown to seedling stage in the greenhouse and indexed on seedling peach in the field. It was demonstrated that at least 10 per cent of the Mahaleb seeds transmitted the ring spot virus and at least 8.7 per cent transmitted the cherry yellows complex. The cherry yellows complex was not transmitted through the seed of Montmorency in these experiments but at least 30 per cent of the seeds carried the ring spot virus.

The foregoing studies are believed to offer the first detailed account of seed transmission of viruses concerned with the cherry yellows complex. They emphasize the need of a disease-free source of seedlings in programs of stone fruit virus certification, particularly for cherry understocks.

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FIELD INFECTION EXPERIMENTS WITH RACES 15A AND 15B OF PUCCINIA GRAMINIS TRITICI[1]

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From the point of view of stem-rust resistance, the great merit of the Hope– and H-44–derivative wheats, now widely grown in the United States and Canada, is that they are resistant in the adult plant stage to all the physiologic races of *Puccinia graminis tritici* Erikss. & Henn. that have been prevalent in the regions in which these varieties are grown. The advent of any physiologic race of more than usual virulence towards these varieties is therefore a matter of practical importance.

The wide interest in race 15B is due to the fact that it was reported more pathogenic than other races to many rust-resistant wheats, including Hope and H-44 derivatives (2, 4). The severe infection of the latter wheats by this race appears to be the first instance in North America of breakdown of the adult-plant resistance of the Hope–H-44 type. Although race 15B has not occurred commonly in the United States or Canada in past years, there is no known reason why it should not at some future time gain wider prevalence than heretofore. In view of the possible future spread of this race, it seemed desirable to determine as accurately as possible, under field conditions, its virulence towards the stem-rust resistant wheats now grown in Canada and, particularly, to compare its pathogenicity with that of race 15A, which has been present in this country for many years. It was also thought desirable to acquire a more precise knowledge of the reaction to these two rust biotypes of wheats now in use as breeding material, such as the Kenya wheats, McMurachy, and Red Egyptian. Tests in the greenhouse at Winnipeg, in 1945, had indicated that race 15B possessed greater pathogenicity than race 15A towards Regent, Redman, Thatcher, and certain other varieties; but, as greenhouse tests can not be regarded as adequate indications of field reaction, it was felt that decisive information of the kind desired could be secured only in field tests. Accordingly, tests were carried out with a group of varieties listed in table 1 during the summers of 1945, 1946, and 1947, in small field plots inoculated with pure cultures of certain accessions of races 15A and 15B. These plots, composed of 5-foot rows surrounded by guard rows of a susceptible wheat, were sown at a distance from each other and from any other plots subjected to artificially produced stem-rust infestation. The plots were sown as early in the spring as practicable so as to escape natural stem-rust infection in so far as possible.

Rust inoculation was performed at time of heading by the method described by Cherewick (1). Briefly, this consisted in dusting each plot as

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uniformly as possible with a spore-talc mixture containing pure inoculum of the physiologic race employed. By using a large supply of inoculum, it was possible to distribute spores evenly over the plot in large enough quantity to produce heavy, uniform infection in minimum time. By this procedure it was possible to take rust readings on these plots before stem-rust infection from other sources became general.

EXPERIMENTAL RESULTS

The data derived from the plot experiments are summarized in table 1

TABLE 1.—*Percentage infection*[a] *produced by different accessions of races 15A and 15B of stem rust and by mixtures of races on certain wheat varieties*

Wheat variety	1945 Race and source[b]			1946 Race and source			1947 Race and source					
	Mixture	15A (C)	15B (M)	Mixture	15A (W)	15B (M)	15A (C)	15A (W)	15A (N)	15A (SC)	15B (M)	15B (K)
Vernal	....	18	55	15	5	70	5	5	15	tr	23	48
Kenya, R. L. 1373	tr	tr	1	0	5	20	tr	tr	tr	1	30	8
Kenya × Gular												
S869	.....	.....	.....	.....	.....	.....	1	1	8	tr	15	30
S871	.....	.....	.....	.....	.....	.....	tr	tr	1	tr	18	35
S872	.....	.....	.....	.....	.....	.....	1	1	6	tr	5	35
Red Egyptian	tr	tr	4	tr	tr	25	tr	tr	tr	tr	20	4
McMurachy	tr	tr	13	tr	tr	20	tr	tr	tr	tr	28	10
R. L. 2327[c]	.....	.....	.....	tr	10	55	6	5	3	1	30	38
Thatcher	8	25	73	40	10	80	13	18	15	2	48	68
Rival	1	28	63	60	tr	70	.....	.....	.....	.....	.....	.....
Regent	tr	2	50	15	10	75	.....	.....	.....	.....	.....	.....
Redman	.....	.....	.....	20	10	75	15	8	5	3	60	70
Hope	tr	.....	.....	.....	.....	.....	1	5	5	5	35	35
Iumillo	tr	tr	20	tr	tr	20	0	0	0	0	1	2
Carleton	.....	.....	.....	50	75	70	.....	.....	.....	.....	.....	.....
Stewart	.....	.....	.....	50	60	75	.....	.....	.....	.....	.....	.....
T. timopheevi	.....	tr	4	tr	0	5	tr	tr	0	0	tr	4
Garnet	.....	.....	.....	85	75	75	90	88	88	90	70	80
Marquis	88	.....	.....	.....	.....	.....	85	85	88	85	75	83

[a] Figures for 1945 and 1947 are averages of readings of two plots.

[b] The mixture of races did not contain race 15B. Sources of accessions are designated as follows: (C)—Cullen, Sask.; (M)—University of Minnesota; (W)—barberry, Winnipeg, Man.; (N)—Normandin, Que.; (SC)—Swift Current, Sask.; (K)—Killarney, Man.

[c] A hybrid line derived from the cross (McMurachy × Exchange) × Redman.

in the form of percentage of infection estimated according to the Cobb scale as modified by the U. S. Department of Agriculture.

Four accessions of race 15A and two of 15B were used. The first accession of race 15B was kindly supplied by Dr. E. C. Stakman, Department of Plant Pathology, University of Minnesota, in the spring of 1945; the second was collected at Killarney, Manitoba, in 1946. The four accessions of race 15A were all of Canadian origin. All of the rust cultures employed had been identified as either race 15A or 15B in greenhouse tests prior to their use in the field experiments.

To compare the severity of infection produced by race 15B with infection produced by a mixture of stem-rust races, inoculations were made simultaneously, except in 1947, with a race-mixture containing nearly forty physiologic races including race 15A but not race 15B.

1945 experiments—Only one accession of race 15A (collected at Cullen, Saskatchewan) and one of 15B (Minnesota accession) were available in 1945. Averages of rust readings of two separate replicates of each of the varieties included in the plots are given in table 1. A comparison of the percentages recorded for the two biotypes shows race 15B to be much more virulent than race 15A towards the varieties Vernal, Thatcher, Rival, Regent, McMurachy, and Iumillo; and somewhat more so towards Red Egyptian and *Triticum timopheevi.*

No yield data were taken but 1000-kernel weights showed that race 15B caused much more shrinkage of kernels than did race 15A in the varieties Rival, Regent, and Thatcher.

The results obtained with a mixture of physiologic races containing all available races except 15B resembled those obtained with race 15A, except that the latter rusted the variety Rival more heavily.

1946 experiments—In 1946, the accession of race 15B from the University of Minnesota was compared, in similar field plot experiments, with a culture of race 15A isolated from aecia collected on a naturally infected barberry at Winnipeg. Two separate plots were infected with each biotype but, owing to the accidental destruction of one set of plots, only one reading on each wheat variety was secured for each biotype. For comparison with the two biotypes, another plot was infected with a mixture of stem-rust races other than 15B.

The infection results for the two biotypes were strikingly different. On the varieties Vernal, Thatcher, Rival, Regent, and Redman, race 15B produced infection recorded as 70 per cent or more, whereas the infection produced by race 15A did not exceed 10 per cent. That these differences were due to the greater virulence of race 15B towards these varieties and were not the result of a generally heavier infection in the plot inoculated by race 15B is shown by the fact that race 15A produced about the same amount of infection as 15B on the durums Carleton and Stewart (60–75 per cent) and on the susceptible wheat Garnet (75 per cent).

The infection results with the mixture of stem-rust races resembled those obtained with race 15A, except that the race-mixture produced heavier infection on Rival and Thatcher.

1947 experiments—In 1947, field plots were infected by two different accessions of race 15B (one from Minnesota and another collected at Killarney, Manitoba, in 1946) and by four Canadian accessions of race 15A.

The infections recorded in table 1 show clearly that both accessions of race 15B were decidedly more pathogenic than any of the cultures of race 15A towards all the varieties tested except the susceptible wheats Garnet and Marquis and possibly the highly resistant *Triticum timopheevi.*

In these tests there are some indications of pathogenic differences between the two accessions of race 15B. The three strains of Kenya × Gular included in the tests were attacked more heavily by the Killarney accession than by the one obtained from Minnesota—a result in agreement with seedling tests. On the contrary, the varieties Kenya, Red Egyptian, and McMurachy were attacked more heavily by the Minnesota accession.

The four accessions of race 15A did not differ greatly in the percentages of infection recorded except that the Swift Current culture produced generally less infection than the others.

DISCUSSION

A study of the data in table 1 will show that the six accessions of wheat stem rust used in the field-plot experiments fall into two groups as to amount of infection caused on the resistant wheats. One of these groups is composed of the four cultures identified in seedling tests in the greenhouse as race 15A; the other is made up of the two cultures identified as race 15B. The most satisfactory of the various seedling infection tests tried thus far for differentiating the two biotypes is the reaction of three hybrid lines of the cross Kenya × Gular. These lines were highly resistant to the cultures designated as race 15A and moderately susceptible to those designated as 15B. The two groups of cultures showed other, though less striking, pathogenic differences, such as a somewhat greater virulence on the part of the two 15B cultures towards seedlings of the varieties Kota, Rival, and Fronteira. The infection results obtained in the field make it clear that the pathogenic characteristics of these rust cultures on seedling plants are also expressed to a considerable degree on adult plants of the same varieties growing in the field. In the varieties Hope, Regent, and Redman, there appears to be no seedling resistance to either biotype of race 15 but, as shown in the field experiments, there is a certain degree of adult-plant resistance to race 15A but not to race 15B.

Although the cultures studied are here designated as either race 15A or race 15B, there are probably slight pathogenic differences among individual cultures of each group. The Killarney accession of the B biotype is somewhat more pathogenic to Kenya × Gular in both the seedling and the adult-plant stages than that from Minnesota and it is possibly slightly more pathogenic to *Triticum timopheevi*. In this connection it is worth noting that Vallega and Favret (5) have recently described a culture of race 15, collected in Argentina, to which *T. timopheevi* was fully susceptible in greenhouse tests.

The results of the field experiments support the conclusion (2) that race 15B is potentially dangerous to the rust resistant wheat varieties now grown in Canada and the United States and they suggest further that this biotype may, under conditions prevailing at Winnipeg, do moderate damage to varieties with stem-rust resistance of the kind possessed by Kenya R.L. 1373, McMurachy, and Red Egyptian.

The amount of infection produced on these three varieties by race 15B (Minnesota accession) was not very consistent from year to year. Kenya R.L. 1373, for example, bore only trace or 1 per cent infection in 1945, but had 20 per cent infection in 1946 and 30 per cent in 1947. That this inconsistency is the result of environmental influences is likely as it has been shown (3) that the stem-rust reaction of both Kenya R.L. 1373 and McMurachy is influenced by temperature. It is worth noting that these varieties react very differently to stem rust in different parts of the world. In Canada they have shown, apart from their reaction to 15B, a high degree of stem-rust resistance. In Kenya Colony, East Africa, they have been classed as stem-rust susceptible wheats,[3] having proved considerably more susceptible than the H-44–derivative Regent.

The amount of infection caused by the two accessions of race 15B used in these experiments compares rather closely with that reported by Hart (2) for a mixture of physiologic races in which the B biotype evidently played a prominent, though not an accurately determinable, part. In the present experiments, 15B was not compared with such a mixture of races but comparisons were made in 1945 and 1946 of race 15B and a mixture of all other available races, including 15A (Table 1). The infection produced by this race-mixture is comparable with the infections usually produced by such mixtures in the artificially-produced stem-rust epidemics on field plots at Winnipeg, although in 1946 it was somewhat heavier than usual on Rival and Thatcher. Race 15B differed from the race-mixture by causing a much heavier infection on Vernal, Kenya, Red Egyptian, McMurachy, R.L. 2327, Regent, Redman, and Iumillo.

Although the B biotype of race 15 has apparently shown little or no increase in prevalence since its discovery in the United States, there is a possibility that it may sooner or later come into prominence. This possibility should be kept in mind by pathologists and breeders concerned with wheat breeding for stem-rust resistance and any future breeding program should include a search for wheats with a specific resistance to this race.

SUMMARY

Four different stem-rust cultures identified by means of seedling infection tests as race 15A and two cultures identified as race 15B were compared for pathogenicity towards a group of wheat varieties in small field plots. Race 15B was decidedly more virulent than race 15A on the stem-rust-resistant common wheats now grown in Canada and on a number of other wheats generally regarded as possessing high stem-rust resistance.

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THE TOXICITY OF POLYSACCHARIDES AND OTHER LARGE MOLECULES TO TOMATO CUTTINGS[1]

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INTRODUCTION

Previous fractionation (10) of a filtrate from cultures of the crown-gall organism, *Phytomonas tumefaciens* (Smith and Townsend) Bergey *et al.*, yielded a wilt-inducing, alcohol-insoluble substance. This consisted largely of the polysaccharide previously isolated both from a virulent (13) and from an attenuated culture (8). The main wilt-inducing component secured by purification of this fraction was a glucosan.

This glucosan contained no specific functional groups thought capable of imparting this unique activity to the molecule. The question arose whether other polysaccharides and also different types of substances of medium molecular weight might induce wilting. Certain of these have been examined for such activity in tomato cuttings. The results both of these tests and of some experiments on the mechanism by which such molecules induce wilting are presented in this paper. Abstracts covering part of this work have appeared already (9, 11).

MATERIALS AND METHODS

The gums and polysaccharides and other polymers were obtained from various sources. Preparations of glucosan from crown-gall bacteria were the same as those previously described (8). Gum from *Rhizobium trifolii* Dangeard was prepared according to the procedure of Hopkins, Peterson, and Fred (12). The polyethylene glycol and Carbowax samples were kindly supplied by the Carbide and Carbon Chemical Company; the samples of polyvinyl alcohol by the E. I. du Pont de Nemours and Company. The other substances were supplied as indicated in table 1 by various investigators to whom the authors express their grateful thanks.

The methods used both in growing the tomato plants and in testing the various preparations were the same as those previously described (10). The condition of the plants used for testing influenced the type and severity of symptoms. Young, succulent cuttings wilted more readily and showed stem and petiole flaccidity more completely than older cuttings.

Unless otherwise stated, the materials were assayed at 0.4 per cent concentration (certain of these showed activity in 0.1 per cent solution), and all samples were adjusted to pH 3.0 before the assay. For each test of a solution, 5 or more cuttings were employed. Each cutting was placed in a gradu-

[1] Supported in part (a) by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation, (b) by the Donner Foundation, and (c) by the American Cancer Society. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.
The illustrations were prepared by Eugene Herrling.

ated test tube containing from 5 to 20 ml. of solution. Tests were made in a room with controlled temperature at approximately 25° C., with relative humidity at about 70 per cent, and with continuous light from a series of fluorescent lamps.

Reducing sugar was determined according to Shaffer and Somogyi (21, Reagent 50 with 5 gm. potassium iodide per liter) and to the micro-colorimetric method of Somogyi (22). Polysaccharide was estimated from the difference in the amount of reducing sugar formed upon hydrolysis with 1 N hydrochloric acid at 100° C. in 10 minutes and in 2 hours. Carbowax compounds were estimated by a modification of the Shaffer and Critchfield (20) method. In the modification the samples were precipitated, washed, digested, and the colorimetric estimations were made in the same tubes. Since the determination was made in a smaller container than that used by the above investigators, *i.e.*, 14×128 mm. Pyrex colorimeter tubes, proportionately smaller quantities of sample and reagents were employed. Colorimetric readings were made with the Klett-Summerson photoelectric colorimeter containing a 520 mμ filter. The range of the assay is from 20 to 80 γ Carbowax per tube.

RESULTS

Activity of various polysaccharides and gums.—Aqueous solutions (0.4 per cent) of various polysaccharides and gums from higher plants and from microorganisms were tested for wilting activity in tomato cuttings. The degree of activity of each substance was estimated, when possible, from the amount of solution absorbed by the cutting and the amount of wilting or injury induced, as previously described by Hodgson *et al.* (10). This method was developed primarily to estimate the extent of wilting when such injury was confined to the leaflets. It could not be used satisfactorily when the stems and petioles particularly were affected.

Preparations from black spruce, *Betacoccus arabinosaceus* Orla-Jensen (*Leuconostoc mesenteroides*), and *Rhizobium trifolii* were assayed once since only limited quantities of the substances were available; all others were assayed at least twice.

All the preparations listed in table 1 wilted tomato cuttings. However, the types of wilting observed, varied. The preparations were divided roughly into two groups depending upon the type of wilting induced, *e.g.*, (a) those affecting primarily the leaflets leaving the stems and petioles turgid (Fig. 1, A and D) and (b) those producing a generalized wilting of the cutting, affecting particularly the petioles and to a lesser extent the stems and leaflets (Fig. 1, B and C).

Certain properties of the preparations appeared to be associated with the type of wilting observed. The substances readily soluble in water and yielding clear, non-opalescent solutions affected primarily the leaflets. Those that were less water-soluble and that yielded opalescent solutions usually affected primarily the petioles and stems.

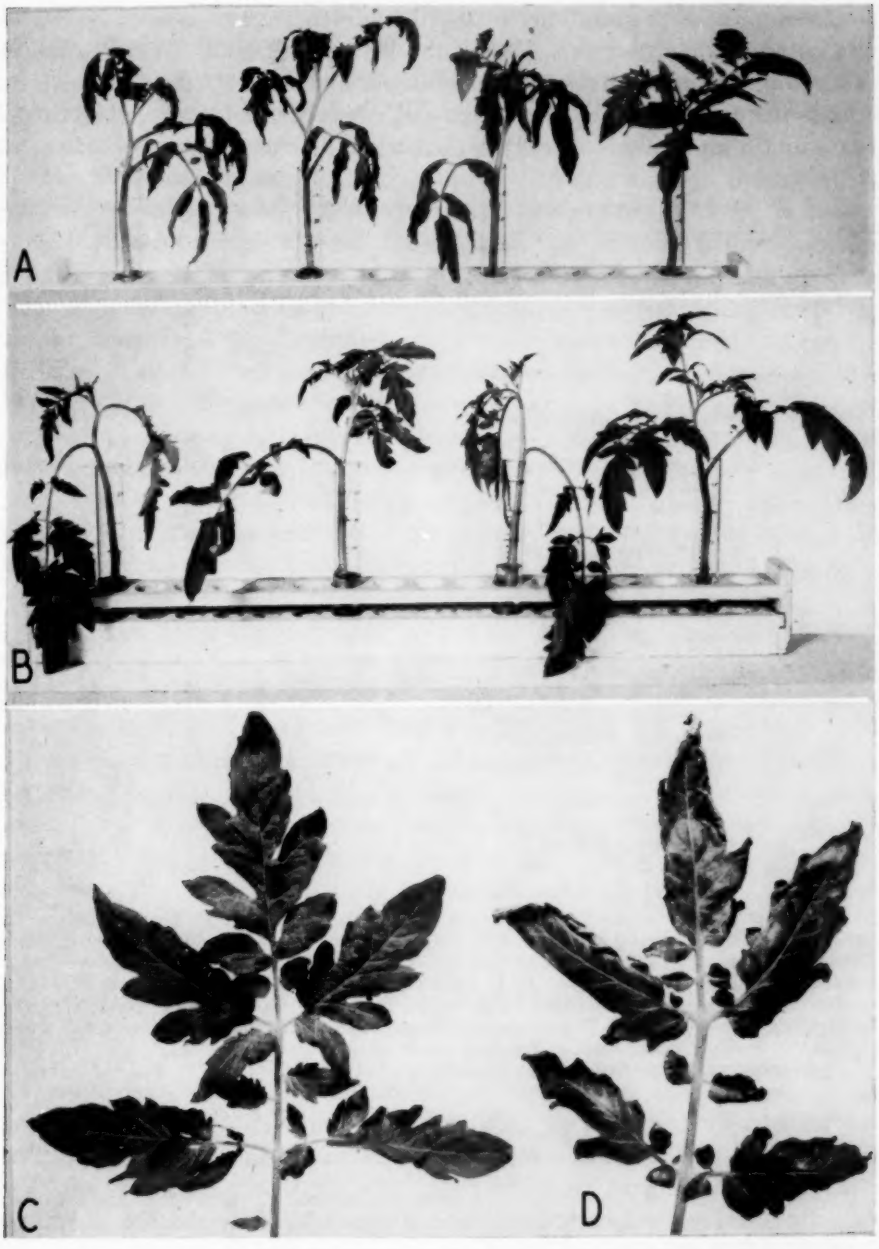

FIG. 1. A. Tomato cuttings after 48 hours, left to right, in solutions of inulin, soluble starch, glucosan from crown-gall bacteria, and distilled water. The first three cuttings are injured severely, as shown in detail in D. B. As A except containing xylan, pectin, agar, and distilled water. The flagging of petioles and stems is conspicuous. However, leaflet injury is slight, as shown in detail in C. C. Representative leaflets from a cutting in a solution of glycogen, a polysaccharide wilting petioles and stems with only slight injury to leaflets. D. As C except from a cutting in a solution of glucosan from crown-gall bacteria, a polysaccharide inducing leaflet wilting and necrosis, but with little effect on petioles and stems.

Among those preparations primarily affecting leaflets, some variation was noted in the syndrome. When the injury was mild, some substances (*e.g.*, glucosan of *Phytomonas tumefaciens* and corn syrup dextrins) induced curling and rolling of the tips and edges of the leaflets, while others (*e.g.*, inulin and soluble starch) caused a collapse of the outer portions of

TABLE 1.—*Wilting activity of polysaccharides and related substances*

Type	Source[a] or name	Approx. mol. wt.	Number of cuttings	Solution intake[b]	Toxic index[b]	Toxic units[b]
				Ml.		*Per gm.*
Compounds acting mainly on leaflets						
Fructosans	*Bacillus subtilis* 1	.....	9	2.2	3.4	390
	Azotobacter indicum 1	.....	9	1.9	2.3	330
	Inulin 2	5,000	15	3.7	4.1	280
Glucosans	Soluble starch 2	4,000	30	3.4	3.6	270
	Phytomonas tumefaciens 3	3,600	20	6.2	2.6	100
	Corn syrup dextrins 4					
	12 glucose units	2,000	16	10.7	1.7	40
	7.5 glucose units	1,200	16	11.2	1.3	30
	5.4 glucose units	1,000	16	11.9	1.2	25
Mixed	Black spruce 5	.....	8	2.9	0.7	60
Compounds acting mainly on petioles						
Glucosans	*Betacoccus arabinosaceus* 6	2,600	5	1.2	.....	.....
	Unidentified bacterium 1	.....	9	1.7	.....	.....
	ß amylase limit dextrin 7	.....	10	1.7	.....	.....
	Leuconostoc dextranicum 1	> 324,000	9	2.2	.....	.....
	Glycogen 2	> 550,000	10	2.6	.....	.....
Mixed	Lemon pectin 8	> 20,000	20	1.6	.....	.....
	Agar 9	.....	25	2.2	.....	.....
	Rhizobium trifolii 3	.....	5	7.4	.....	.....
Pentosan	Xylan 2	> 10,000	20	2.1	.....	.....

[a] The sources of these substances were as follows: 1—Dr. J. P. Martin, Citrus Experiment Station, Riverside (described by Martin, 14); 2—Purchased from the Pfanstiehl Chemical Company; 3—Prepared in this laboratory (see text); 4—Dr. Dexter French, Iowa State College, Ames; 5—Dr. F. E. Brauns, Institute of Paper Chemistry, Appleton; 6—Dr. W. Z. Hassid, University of California, Berkeley; 7—Dr. W. J. Olson, Malting Laboratory, University of Wisconsin, Madison; 8—Purchased from Eastman Kodak Company; 9—Purchased from Difco Laboratories.

[b] Average value for the number of cuttings indicated.

the leaflet leaving turgid an area around the midvein. Under severe conditions (*e.g.*, by the use of a more concentrated solution), in both types the whole leaflet was often completely dry and necrotic. Such differences in symptoms did not appear related to the type of polysaccharide or linkage therein. Thus fructosans, glucosans, pentosans, and polysaccharides with mixed sugars all affected the leaflets.

The toxic unit value is defined as the product of sample dilution and toxic index divided by solution intake in milliliters (10). Thus a toxic unit is the amount of substance dissolved in 1 ml. of a solution that caused a toxic index of 1. In general the toxic unit is large when the solution intake is low.

However, some preparations (*e.g.*, black spruce polysaccharide) do not cause severe symptoms even though the solution intake is low.

The toxic unit value (Table 1) for glucosan from crown-gall bacteria is lower than previously reported (10). This probably is due to the use of somewhat larger cuttings in the present work.

The molecular weight of certain of these preparations was considered in relation to wilt-inducing ability. Unfortunately, it is difficult to obtain suitable samples of known molecular weights. Many polymers are not homogeneous but contain a mixture of molecular species that differ many fold in molecular weight. For many of the samples obtained the molecular weight is not known, and for others only an average molecular weight is known. Various reports[2] give molecular weight data on several of the types of substances tested. A molecular weight determination on each sample was beyond the scope of this work. Hence it is not known how closely the polymers tested correspond to those reported in the literature. However, the data in the upper part of table 1 suggest in general a direct relationship between molecular size and wilt-inducing activity on leaflets. As discussed later, the data suggest that the toxicity of these substances is due to a mechanical interference with the moisture relations of the plant.

Among the preparations causing stem and petiole wilting, the molecular weights of only a few are known.[3] As explained above no adequate means had been evolved for estimating the potency of these substances; hence a molecular weight-toxicity relationship was not readily demonstrated.

The amount of solution taken up by the cuttings presumably determines the actual weight of substance absorbed. Considerable variation was thus noted in the quantity of different substances taken into the plant during the 48-hour test period. This amount of substance is involved in the toxic unit value for those compounds having activity on leaflets. A smaller

[2] Glucosan from *P. tumefaciens* has a molecular weight of about 3,600 (13). Harvey (4) lists inulin as 5,000 and soluble starch as about 4,000; however, our samples may have been different. The corn syrup dextrins were approximately 2,000, 1,200, and 1,000, respectively, for the 12 unit, 7.5 unit, and 5.4 unit molecules.

[3] The polysaccharide of *Betacoccus arabinosaceus* has a molecular weight of about 2,600 (5). It might be expected that this substance would cause wilting of leaflets. However, its low solubility in water may account for its wilting behavior on petioles and stems. Peat, Schlüchterer, and Stacey (17), from osmotic pressure measurements of a dextran produced by a strain of *Leuconostoc dextranicum (Betacoccus arabinosaceus haemolyticus)*, estimated a minimum chain length of 200 glucose units (molecular weight about 324,000). Methylated glycogen preparations from various sources were found by Carter and Record (1) to have chain lengths of 3,400 to 5,400 glucose units (molecular weights about 551,000 to 875,000) as measured by osmotic pressure. Oakley and Young (16), from osmotic pressure measurements, found rabbit liver glycogen to have a mean particle weight of the order of 2,000,000. Various pectin preparations may have molecular weights ranging from less than 30,000 to over 200,000, as indicated by Schneider and Fritschi (19) and by Speiser and Eddy (23). For example, the latter investigators fractionated a sample of nitrated pectin having an average molecular weight of 125,000. Molecular weight species ranging from 20,800 to 268,000 were obtained. The pectin tested in the present work induced slight wilting of leaflets and also of petioles. The leaflet symptoms may have been caused by the smaller molecules and the petiole symptoms by the larger ones. From viscosity measurements Haworth (7) estimated that xylan may have a chain length of 75 to 80 pentose units (molecular weight about 10,000 to 10,500).

quantity of substance having the higher toxic unit value was necessary to cause a similar toxic index.

Considerable variation also was noted in the amount of solution taken up by cuttings in solutions of substances causing wilting of petioles and stems. Thus about 4 mg. of polysaccharide of *Betacoccus arabinosaceus* and about 29 mg. of polysaccharide of *Rhizobium trifolii* apparently were absorbed per plant. In general less of the substances causing petiole and stem wilting was absorbed in the 48-hour test period than of those causing leaflet wilting.

Undoubtedly the shape of the molecule also is important. A molecule with short cross section would be expected to pass through a membrane perhaps more rapidly than one of large cross section when the pore size was limiting. However, the difficulty of obtaining suitable compounds prevented a study of this factor at present.

Activity of other polymers.—More data were sought on the suggested relationship between molecular weight and wilt-inducing activity. The polyethylene glycols were used since they are available in several molecular weight ranges. They are readily soluble in water and are reported to have low toxicity to plants (3, 15).

The polyethylene glycols have the general formula $HOCH_2\ (CH_2OCH_2)_x\ CH_2OH$ where X varies with the molecular weight of the compound. Those with average molecular weights varying from 200 to 700 are liquids; those above 1000 are waxy solids called "Carbowax". For this work ethylene glycol, polyethylene glycols 200 and 400, and Carbowaxes 1540, 4000, 6000, and 9000 were employed. The number of each preparation corresponds roughly to the average molecular weight. However, the molecular weight of Carbowax 4000 has been estimated as 3,000 and 3,590, respectively, by independent methods (20). It is not known how closely the molecular weights of the other compounds actually conformed to their designated numbers.

Aqueous solutions (0.1 to 0.4 per cent) of several of these compounds were tested in the usual manner. Certain of them induced severe wilting of tomato cuttings. The symptoms were confined to the leaflets and were similar to those shown in figure 1, A and D. Ethylene glycol alone had mild activity. Polyethylene glycol 200 had somewhat greater activity but polyethylene glycol 400 had negligible activity. Carbowax 1540 induced a severe wilting of tips and edges of the leaflets; in some cases a pronounced uprolling of the edges and irregular, discolored, shiny areas appeared. The symptoms from Carbowax 4000 were like those from Carbowax 1540, but they were more severe. The symptoms from Carbowax 6000 were somewhat like the above. However, no necrotic areas and only a little leaf rolling appeared. Instead, the leaflet edges collapsed from the midvein and only a small area around the midvein remained turgid. Symptoms from Carbowax 9000 were similar to those of Carbowax 6000 but more severe. If the reported molecular weights of the various Carbowax compounds are

plotted against their wilt-inducing activities, in general the points fall along a straight line. This result was obtained in several trials. However, the slope of the line varied considerably from one trial to another. A representative experiment is shown in figure 2. The compound with the highest molecular weight is the most toxic. The behavior of the polyethylene glycols is thus similar to that of polysaccharides in that toxicity in general varies directly with molecular weight.

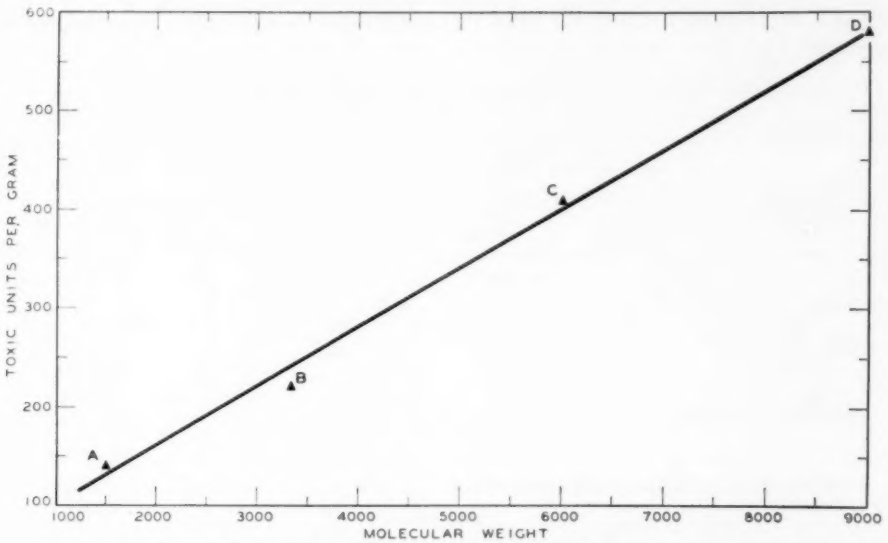

FIG. 2. Relationship of wilt-inducing activity of Carbowax compounds (0.2 per cent solution) to molecular weight. Each point is the average of 5 cuttings. A. Carbowax 1540; B. Carbowax 4000; C. Carbowax 6000; and D. Carbowax 9000.

Another water-soluble polymer used was polyvinyl alcohol (Elvanol) which has been reported to be non-toxic to plants (24). Partially hydrolyzed types contain varying amounts of polyvinyl acetate in addition to polyvinyl alcohol; completely hydrolyzed types contain only polyvinyl alcohol. Both partially and completely hydrolyzed types are available in each of three viscosity ranges, *i.e.*, low, medium, and high. For this work samples were obtained of the completely hydrolyzed type in each of the viscosity ranges. The approximate molecular weight ranges of these are 11,500, 40,000, and 52,000, respectively.

Aqueous solutions (0.2 per cent concentration) were tested on tomato cuttings in the usual manner. The low viscosity type was somewhat less toxic, and the medium viscosity type was somewhat more toxic than Carbowax 6000. Wilting induced by the low and medium viscosity types was confined mainly to the leaflets (similar to those shown in figure 1, A and D). The low viscosity sample induced severe rolling and wilting of tips and edges of the leaflets. That having medium viscosity caused severe wilting of leaflet edges with some rolling and leaflet collapse. That having

high viscosity differed from the above. Only slight leaf rolling appeared, but pronounced flagging of petioles and to a lesser extent the stems and leaflets was apparent (similar to symptoms in figure 1, B and C).

Again the relationship of molecular weight to wilt-inducing activity was evident. The potency of the medium viscosity type was considerably higher than that of the low viscosity type. Unfortunately the potency of the high viscosity type could not be estimated by this method since it induced a very different type of wilting. However, in general, with the two preparations a direct relationship appears between molecular weight and wilt-inducing power.

TABLE 2.—*Action of tomato leaf juice on soluble starch and glucosan from crown-gall bacteria*

Addition to plant juice[a]	Incubation time before heating[b]	Weight of polysaccharide added	Reducing sugar found[c]	Increase in sugar on incubation	Degree of hydrolysis
	Hr.	*Mg.*	*Mg.*	*Mg.*	*Per cent*
None	0	0	42	.....	.....
Do.	72	0	46	4	.....
Soluble starch	0	40	48	.....	.....
Do.	72	40	70	22	45
Glucosan from crown-gall bacteria	0	40	45	.....	.....
Do.	72	40	50	5	3

[a] The reaction mixture contained 10 ml. leaf juice, 10 ml. carbohydrate solution, and 2 ml. toluene.
[b] The heating time was 5 minutes at 100° C.
[c] Estimated as glucose by the micro-colorimetric method of Somogyi (22).

A few experiments were made with nitrogenous substances; *viz.*, filtered egg albumin, peptone, and yeast extract solutions. All these substances caused severe wilting of the leaflets of tomato cuttings (similar to those shown in figure 1, A and D). It is thus apparent that the wilting described is a nonspecific phenomenon which may be caused by a wide variety of substances.

Fate of the toxic substances in treated cuttings.—No entirely satisfactory explanation for the toxic effects of all the compounds tested has arisen. However, a few possibilities were explored.

It might be expected that a polysaccharide such as starch, which is a normal plant constituent and yet induces wilting, would be hydrolyzed readily by plant enzymes. However, a polysaccharide with β-glucosidic linkages, such as the glucosan from crown-gall bacteria, probably would not be readily attacked. These possibilities were examined.

Solutions of soluble starch and glucosan from crown-gall bacteria were incubated with crude plant juice expressed by pressure (6,000 to 12,000 lb. per sq. in.) from tomato leaves. After incubation for 72 hours at room temperature, reducing sugar was determined on each sample. The data (Table 2) indicated that about 45 per cent of the soluble starch, but only

3 per cent of the glucosan from crown-gall bacteria, was converted into reducing sugars. These data indicated a hydrolytic action of the plant juice upon starch. However, such action, if any, on the polysaccharide from crown-gall bacteria was slight.

The possibility was examined that hydrolysis of soluble starch might result in a toxic concentration of sugar. Leaves clipped from cuttings that had stood 48 hours in 1 per cent solutions of (a) soluble starch, (b) polysaccharide from crown-gall bacteria, (c) glucose, (d) sucrose, and (e) distilled water were dried at approximately 85° C. and ground to pass a 60-mesh sieve. Samples (about 0.5 gm.) were weighed into 50-ml. Erlenmeyer flasks, and 5 ml. of distilled water and 5 ml. of 1 N sulfuric acid were added. The mixtures were heated for 10 minutes at 100° C., were filtered, neutralized, and analyzed for reducing sugar.

TABLE 3.—*The sugar content of leaves from tomato cuttings in water and in carbohydrate solutions.*

Solution	Solution intake[a]	Toxic index[a]	Reducing sugar[b]
	Ml.		*Per cent*
Soluble starch	1.0	4.7	3.7
Crown-gall polysaccharide	2.5	2.6	3.2
Glucose	5.6	0.2	6.2
Sucrose	6.2	0.0	7.4
Water	12.3	0.0	2.4

[a] Each value is the average of 5 cuttings.
[b] Sugar values were calculated as glucose on the dry weight basis.

The results (Table 3) indicated that sugar concentration was not the reason for the toxic activity of polysaccharides since sucrose- or glucose-treated plants, which showed no toxic symptoms, contained more sugar than the polysaccharide-treated plants, which had severe symptoms.

The distribution of polysaccharide from crown-gall bacteria was studied in treated cuttings. Hydrolysis (1 N hydrochloric acid at 100° C. for 2 hours) completely degrades this substance into glucose. However, very little glucose is formed from the substance in 10 minutes at 70° C., although sucrose is completely hydrolyzed in this time. Therefore, a rough measure of polysaccharide may be obtained by the difference in reducing sugar formed upon hydrolysis for 10 minutes and 2 hours. Of course, many other substances in the plant (*e.g.*, starch and hemicelluloses) would give rise to reducing sugar when subjected to such treatment. However, an approximate correction for such substances could be made by including appropriate controls.

Leaflets of tomato cuttings that had stood for 24 hours in various solutions were sectioned into wilted and non-wilted portions. These were dried, ground, hydrolyzed and filtered, and the filtrates were analyzed for reducing sugar. The results appear in table 4. The leaves of cuttings placed in

filtrate from crown-gall bacteria contained a greater quantity of polysaccharide than those from cuttings in either water or in the regular sucrose, urea, mineral-salts medium used for culturing the crown-gall organism. With the bacterial filtrate a greater quantity of polysaccharide was found in wilted than in non-wilted portions of the leaflets. As might be expected, the wilted portions of the leaflets had a lower moisture content than the non-wilted portions.

TABLE 4.—*Analyses of normal and wilted tomato leaflets for polysaccharide*[a]

Material	Moisture	Dry weight	Polysaccharide
	Per cent	*Gm.*	*Per cent*[b]
Normal leaflets of cuttings in Tap water			
Centers	93	0.90	4.3
Edges	92	0.94	4.2
Unfermented medium			
Centers	92	1.15	3.4
Edges	90	1.19	3.2
Wilted leaflets of cuttings in Bacterial filtrate			
Centers	91	0.77	10.3
Edges	83	1.42	13.4

[a] Analyses were made on composite samples of 18 cuttings.
[b] Estimated as percentage of the dry weight.

Attempts were made to account quantitatively for soluble starch taken up by cuttings. Analyses for starch were made by the method of Hassid *et al.* (6) on treated cuttings sectioned into stems, petioles, wilted portions of leaflets, and non-wilted portions of leaflets. However, it was possible to account for only a portion (about 30 per cent) of the starch absorbed.

The distribution of the starch found varied with the particular preparation of soluble starch used. One sample was readily soluble in water, formed a clear solution and contained about 12 per cent of reducing sugar. Of that recovered, 49, 14, 9, and 27 per cent, respectively, were found in leaflet edges (wilted), leaflet centers (non-wilted), petioles, and stems. Another preparation of soluble starch was much less soluble in water, formed an opalescent solution, and contained no reducing sugar. Of the amount of this preparation recovered, 13, 16, 4, and 67 per cent, respectively, were found in leaflet edges (wilted), leaflet centers (not wilted), petioles, and stems. In this case most of the starch was in the stems whereas with another preparation most was in the leaflets. Possibly the particle size was too large to reach the leaf margins.

The low recoveries of absorbed starch probably were related to the low light intensity maintained in the test room. Under these conditions the cuttings probably were not synthesizing starch to any great extent, but were converting it into other substances.

A study was made of the distribution of polyethylene glycols in tomato cuttings that were treated with various glycol solutions. After 48 hours in

the solutions the cuttings were dissected into wilted and non-wilted parts of leaflets, petioles, and stems. Composites of several cuttings (usually 5) were dried at 75° to 90° C. in a stream of air, were ground to pass a 60-mesh sieve, and were analyzed for the respective glycols. Representative results are summarized in tables 5 and 6. The following items are particularly noteworthy.

While the toxic index remained somewhat similar, solution intake decreased rapidly with increasing molecular weight and, correspondingly, less of the large molecular-weight substance actually was absorbed by the cutting. Thus, about 18 mg. Carbowax 4000, but only about 6 mg. Carbowax 9000 were absorbed per plant.

TABLE 5.—*Analyses of tomato cuttings treated with Carbowax compounds*

Solutions	Solution intake[a]	Toxic index[a]	Carbowax absorbed[b]	Recovery of absorbed Carbowax[c]
	Ml.		*Mg.*	*Per cent*
Carbowax 1540[d]	11.7	3.2	.....	.....
Carbowax 4000	9.2	4.0	92	97
Carbowax 6000	3.9	3.2	39	87
Carbowax 9000	2.9	3.3	29	94
Distilled water	24.6	0.0	0	.....

[a] Average value from 5 cuttings.
[b] Estimated from the total intake of 5 cuttings.
[c] Values corrected empirically for the "Carbowax" in control tissue.
[d] Composite of 4 cuttings, others composite of 5 cuttings.

Non-wilted portions of leaflets, petioles, and stems of all cuttings were very similar in moisture content. The moisture content of stems and petioles appears somewhat high. This probably is due to water soaking during the 48-hour test period, when the stems were standing in solutions from one to several inches deep.

The distribution of Carbowax in the various parts (calculated as a percentage of the total amount found in the cutting) is not correlated strictly with molecular weight of the particular Carbowax. However, if values for Carbowax 1540 and 4000 on one hand, and Carbowax 6000 and 9000 on the other, are considered, less glycol is present in the wilted portions of leaflets and more in the non-wilted portions with the larger molecular weight substances. This division also corresponds roughly to a subtle difference in symptoms induced, as previously mentioned, *i.e.*, Carbowax 1540 and 4000 have greater activity on leaflet edges than Carbowax 6000 and 9000.

An over-all recovery of 87 to 97 per cent of the glycol absorbed was obtained, and 81 to 91 per cent of the glycol was found in the leaflets. More Carbowax was found in wilted than in non-wilted portions of leaflets. In these cases also there is an accumulation of the wilt-inducing agent in the leaflets.

TABLE 6.—*Distribution of absorbed Carbowax in tomato cuttings*

Part analyzed	Moisture in wet tissue	Wt. dry tissue	Carbowax found[a]	Distribution of Carbowax[b]
	Per cent	*Gm.*	*Per cent*	*Per cent*
Carbowax 1540				
Leaflet edges (wilted)	72	0.63	10.8	68
Leaflet centers (non-wilted)	90	0.46	5.0	23
Petioles	96	0.22	0.7	1
Stems	97	0.48	1.7	8
Carbowax 4000				
Leaflet edges (wilted)	65	1.23	5.6	71
Leaflet centers (non-wilted)	89	0.51	3.2	17
Petioles	96	0.38	0.6	2
Stems	97	0.83	1.2	10
Carbowax 6000				
Leaflet edges (wilted)	81	0.89	2.5	54
Leaflet centers (non-wilted)	89	0.59	1.9	27
Petioles	96	0.33	0.8	7
Stems	97	0.66	0.7	12
Carbowax 9000				
Leaflet edges (wilted)	80	0.89	2.2	59
Leaflet centers (non-wilted)	89	0.57	1.9	31
Petioles	96	0.33	0.1	1
Stems	97	0.60	0.5	9
Distilled water[c]				
Leaflet edges (non-wilted)	90	0.79	0.2[d]	....
Leaflet centers (non-wilted)	92	0.71	0.3[d]	....
Petioles	96	0.34	0.2[e]	....
Stems	97	0.84	0.4[e]	....

[a] Calculated on the dry weight basis.
[b] Calculated as percentage of the total Carbowax found in the whole cuttings.
[c] A small amount of unknown substance in control tissue assays as Carbowax.
[d] Estimated as Carbowax 1540.
[e] Estimated as Carbowax 4000.

DISCUSSION

Among the polysaccharides and gums tested, two general types of wilting symptoms were observed. Some wilted principally the leaflets, others wilted principally the petioles and stems. In addition to polysaccharides, several other types of molecules, *e.g.*, polyethylene glycols, polyvinyl alcohols, and nitrogenous substances, caused wilting primarily of the leaflets. Before any conclusions can be drawn as to the reason or reasons such a wide variety of substances caused similar wilting symptoms in leaflets, several relevant factors should be considered.

Moisture intake was related to the toxicity of a given substance. It should be emphasized that the wilt-inducing potency of a given preparation was estimated from the degree of wilting, the amount of solution absorbed by the cutting, and the concentration of the assay solution, as previously described (10). Thus an extremely potent wilt-inducing substance is one that at low concentration causes considerable wilting and allows only slight moisture intake. It appears that low moisture intake is either the result or the cause of wilting.

A structural consideration of several compounds tested, *e.g.*, the polysac-

charide of crown-gall bacteria, indicates no especially reactive functional groups, such as carboxyls, that might be expected to impart toxicity to the molecule. If toxicity could be assigned to such a functional group, the potency of the compound would be expected to be directly related to its molar concentration. However, among the members of each group of the wilt-inducing substances tested, when the molecular weight was reasonably clear, the largest molecules were also the most toxic. Since all substances were prepared for assay on a weight basis rather than on a molar basis, solutions of the large molecular weight substances contained fewer molecules per unit volume than those of low molecular weight. Yet the solution containing the fewer molecules had greater wilting potency. The compounds having large molecular weights thus appear more toxic simply because they are larger in physical dimensions.

The best explanation as yet available from the evidence is that the various compounds tested induce wilting because they mechanically interfere with the transpiration system of the plant. The exact locus or loci of such an interference are not clear and cannot be assigned on the basis of present information. However, the analytical data indicate that many of the substances, *e.g.*, Carbowax compounds, pass through the large vessels of the stem and petiole and accumulate mainly in the leaflets, particularly in the margins. A mechanical blocking of the transpiration stream possibly at the ends of the tiny veinlets might account for the greater toxicity of the larger molecules. Because of their physical size, the large molecules might be more effective than smaller ones in blocking movement of solutions through a membrane.

Furthermore, the possibility remains that some of these substances actually might diffuse through the cell membrane or pass through the plasmodesma and accumulate within the cells. In such cases they perhaps might interfere with the activity of cell contents.

That soluble starch, a normal plant constituent, should cause wilting even though amylases are present in the leaves, deserves comment. Probably the plant amylases are located principally within the cells rather than in the vessels. Then in treated cuttings such enzymes would have little contact with the starch until it entered the cells. Passage of a moderately large molecule through the cell membrane at best probably would be slow. Thus it appears that large molecules that are sufficiently soluble to be transported in the plant sap and that are too large to move rapidly into the cells might pile up in the ends of the tiny veinlets.

Polysaccharide of crown-gall bacteria in aqueous solution diffused slowly through a cellophane membrane. Since it apparently is not hydrolyzed to any appreciable extent by tomato leaf juice, it might also induce wilting even if it passed readily from the vessels into the cells and accumulated there rather than in the ends of the tiny veinlets.

The present work indicates that the Carbowax compounds are toxic in low concentration when introduced into the vascular system of tomato cuttings.

Concentrations of 0.1 per cent of several Carbowax compounds induced marked symptoms within 48 hours. However, as surface applications these compounds are less toxic. Mitchell and Hamner (15) tested various concentrations of Carbowax in aqueous solution on several plants. Tomato plants were not injured with 1 per cent solutions. Withrow and Howlett (24) reported that undiluted Carbowax 1500 and Carbowax 4000 were very toxic to tomato leaf tissues and flowers. However, none of the leaf tissues of several plants were injured with 1 per cent or less of Carbowax in aqueous solution. On the other hand, tomato floral parts were injured by as little as 0.1 per cent Carbowax.

Certain compounds of the types studied, or related to these, are produced by plant pathogens and have been shown to induce wilting in plants. The polysaccharide of the crown-gall organism induces a wilting and necrosis of tomato leaflets (10). The organism causing the Dutch elm disease has been shown by Dimond (2) to produce (a) a substance that appears to be a polysaccharide and that causes curling of leaflet edges and (b) an unidentified substance causing severe necrosis. Plattner and Clauson-Kaas (18) isolated from a fusarium culture, a peptide which induced wilting similar in appearance to that caused by the glucosan from crown-gall bacteria.

Apparently many organisms produce substances of moderate molecular size. The possibility deserves further study that such metabolites may have important effects largely through mechanical interference in the large vessels, in the small vein islets, or inside the cells.

SUMMARY

The wilt-inducing properties of various groups of polymers (in 0.1 to 0.4 per cent aqueous solution) upon tomato cuttings have been studied under controlled conditions of light, temperature, and relative humidity.

Various polysaccharides of both plant and microbial origin induced wilting in tomato cuttings. Those that were water-soluble and formed clear solutions induced wilting of the leaflets. One of these was shown to accumulate in the leaflets and especially in their margins. Those less soluble and forming opalescent solutions usually caused flaccidity, particularly of petioles and stems. Certain of these apparently caused plugging at the base of the stem. Among those that caused leaflet wilting a direct relationship, in general, appeared between molecular weight and wilt-inducing ability.

Among the polyethylene glycols, Carbowaxes 1540, 4000, 6000, and 9000 were studied particularly. These all caused wilting of the leaflets. A direct correlation again appeared between wilt-inducing potency and molecular weight. Chemical analyses indicated that 81 to 91 per cent of the compound absorbed accumulated in the leaflets, with the highest concentration appearing in the wilted portion.

Among the polyvinyl alcohols, those of low and medium molecular weights, *e.g.*, 11,500 and 40,000, induced wilting primarily of leaflets. A

third compound (molecular weight about 52,000) caused flaccidity, particularly of petioles and stems. A direct relationship between molecular weight and toxicity again appeared with those preparations causing leaflet wilting.

The data obtained indicated that the wilting induced by these polymers may be correlated with molecular weight. Toxicity could not be assigned to any functional group common to such a diverse group of substances. The data seemed best explained by assuming that these substances were toxic because they mechanically interfered with the transpiration system. While the loci of such an interference could not be assigned on the basis of present evidence, it seemed possible that these substances might accumulate, for example, in the large vessels at the ends of the vein islets, or inside the cells, depending in large part on their size.

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SELECTION AND PROPAGATION OF ALBIZZIA FOR RESISTANCE TO FUSARIUM WILT

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The mimosa tree (*Albizzia julibrissin* Durazz.) is used extensively as an ornamental in southern United States, where it is valued for its rapid growth, graceful foliage, and colorful flowers. A destructive vascular wilt disease, caused by *Fusarium oxysporum* f. *perniciosum* (Hepting) Toole (1, 4, 6), presents a threat to the future of this tree in the country.

The selection and propagation of lines of the mimosa tree resistant to the wilt offered a possible solution to the problem of control. The control of Fusarium wilts through soil treatments and amendments has not proved generally practicable, and even if successful, such treatments may be expensive or a nuisance when applied over the life of a tree. Results are reported herein of experiments carried on since 1939, to discover and propagate wilt-resistant mimosas. Some of the early efforts along these lines have already been briefly reported (2, 3).

TESTING OF SEEDLINGS FOR WILT-RESISTANCE

Although careful watch had been kept for mimosa trees resistant to or immune from the Fusarium wilt in some of the heavily wilt-infested areas, no such trees were found. Therefore, tests were made of the susceptibility to wilt of seedlings from a large number of trees chosen at random over much of the range of the mimosa in the southern states. Seed was obtained in the fall of 1939, from 50 trees of *Albizzia julibrissin* scattered from Maryland to Louisiana, 1 tree of *A. julibrissin* var. *rosea* (Carr.) Mouillef. from Jamaica Plains, Mass., and 2 trees of *A. kalkora* (Roxb.) Prain., 1 each from Savannah, Ga., and Biltmore, N. C.

In the first experiment (Table 1) seedlings from these seed lots, grown in flats in the greenhouse during the winter of 1939–40, were tested for resistance in the spring of 1940 by inoculation in the greenhouse, with highly virulent strains of the wilt *Fusarium*, by procedures previously described (6). All the seedlings from most parent seed lots of *Albizzia julibrissin* died of wilt by the end of the 1940 growing season. However, some seedlings in a few of these groups, together with several seedlings of *A. julibrissin* var. *rosea* and *A. kalkora*, remained healthy. These survivals were given a second inoculation in the greenhouse in the spring of 1941, and the 30 seedlings still alive the following season were inoculated a third time and planted in naturally wilt-infested soil near Tryon, N. C.

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In a second experiment (Table 1) additional seedlings were grown from the seed of the 4 parent trees that yielded the most resistant progeny in 1940. These, together with seedlings from a susceptible tree, were inoculated in the spring of 1941. The 64 surviving seedlings were inoculated a second time in 1942 and planted in naturally wilt-infested soil.

Many of the trees set out at Tryon in 1942 died within a month or two after they were transplanted, because of the extremely dry season, but they

TABLE 1.—*Summary of inoculations on selections of mimosa seedlings*

Experiment[a]	Species of Albizzia	Parent seed lots	Original seedlings inoculated	Trees alive in 1948
Number		*Number*	*Number*	*Number*
1	*A. julibrissin*	2	15	5
	A. julibrissin	48	444	0
	A. julibrissin var. *rosea*	1	9	1
	A. kalkora	2	20	2
2	*A. julibrissin*	2	64	12
	A. julibrissin	3	80	0
3	*A. julibrissin*	10	336	203
	A. julibrissin	9	317	0
	A. julibrissin var. *rosea*	2	7	6
	A. kalkora	2	47	43
4	*A. julibrissin*	4	98	55

[a] The seedlings in experiment 1 were inoculated in 1940, 1941, and 1942; those in experiment 2 were inoculated in 1941 and 1942, and those in experiments 3 and 4 were inoculated in 1947.

had no symptoms of Fusarium wilt. A few others were accidentally mowed down. Twenty trees from these first two experiments, representing 4 different parent trees of *Albizzia julibrissin* and 1 each of *A. julibrissin* var. *rosea* and *A. kalkora* have remained alive through six growing seasons in the field (Table 1), while neighboring volunteers have died of the wilt disease. These 20 trees unquestionably possess some degree of resistance to mimosa wilt.

In experiment 3 (Table 1), started in 1946, the material grown and tested for resistance comprised seedlings from certain lots of 1939 seed that yielded resistant trees in experiments 1 and 2; seedlings from 1946 seed from these same trees; and seedlings from 1946 seed from a number of untested trees. They were inoculated in the spring of 1947, and at the end of the first season important differences in resistance were apparent (Table 1). The parent trees which yielded resistant progeny in earlier tests, using the original 1939 seed, again gave a high percentage of resistant seedlings; also, new collections from these trees gave additional resistant seedlings; and additional resistant parent trees were discovered from the 1947 tests.

In 1946, several of the 6- and 7-year-old resistant trees discovered in the first trials yielded seed for the first time. These seeds were collected

and seedlings from them were tested by inoculation in the greenhouse in 1947 (Experiment 4, Table 1). Fifty-six per cent of these seedlings survived one inoculation, compared with no survivals among seedlings from randomly selected trees. With uncontrolled pollination, it is obvious that progeny from resistant trees cannot be depended upon for resistance.

VEGETATIVE PROPAGATION

Because wilt-resistant trees do not pass on the factor for resistance to all of their seedling progeny, it becomes necessary to propagate these trees vegetatively to increase the quantity of resistant stock. The remainder of this paper reports the results of methods of propagation of the mimosa by means of cuttings and testing them by inoculation.

Hardwood Stem Cuttings

Experiments were started to determine ways of rooting mimosa cuttings, without regard to wilt resistance. The resistant trees could not provide adequate cutting material and so hardwood stem cuttings were taken from wildling mimosa trees. A group of young vigorous trees growing near Asheville, N. C., was selected for stock material, and on March 24, 1945, shoots several feet long were cut from these trees and placed in water. Soon after collection the shoots were cut into sticks approximately 8 inches long and a record was made of their basal diameters. Twelve cuttings were available for each of 6 treatments. These treatments included dipping the lower ends in: (1) tap water for 20 hours; (2 and 3) indolebutyric acid at 40 p.p.m. and 500 p.p.m., respectively, for 20 hours; (4 and 5) naphthaleneacetic acid at 40 p.p.m. and 500 p.p.m., respectively, for 20 hours; and (6) 2-4-D at 50 p.p.m. for 1 hour. The upper ends of 6 cuttings in each treatment were dipped in wax, while the other 6 were not waxed. At the end of the treatment period, all cuttings were rinsed in tap water and planted in moist sand in flats in the greenhouse.

Four months after planting, all cuttings were dug and examined. Those that had not rooted were dead. Rooting had occurred only in cuttings that were treated with indolebutyric acid at 40 p.p.m., naphthaleneacetic acid at 40 p.p.m., or 2-4-D at 50 p.p.m. With one exception, cuttings less than 0.3 inch in diameter had not rooted. Rooting of larger cuttings was aided by waxing the tops: All the larger, top-waxed cuttings treated with indolebutyric acid at 40 p.p.m. rooted, whereas only 50 per cent of similar unwaxed cuttings rooted. Cuttings treated with indolebutyric acid are in figure 1.

In the spring of 1946, another experiment was undertaken, to compare the rooting of stem cuttings from resistant versus random wildling trees, and to provide a source of cuttings for inoculation tests comparing cuttings from these sources. Shoots were used from 6 of the resistant mimosa trees at Tryon, N. C., 3 nearby wildling trees, and 3 wildling trees from

Asheville, N. C. Two vigorous shoots from each tree were cut on March 11, 1946, and placed in water. The next day they were cut into 8-inch lengths, the upper ends waxed, and the bases set in a solution containing 40 p.p.m. of indolebutyric acid. After 24 hours, these cuttings were rinsed and planted in sand flats in the greenhouse. They were dug one year later. Four per cent of the cuttings from one resistant tree had rooted, 50 per

FIG. 1. Hardwood mimosa cuttings one-fifth natural size, 4 months after treatment [with indolebutyric acid, 40 p.p.m. for 20 hours.] The two unrooted cuttings on the left are under 0.3 inch in diameter, and are dead.

cent from another, and none from the remaining 4. The Asheville wildling cuttings and cuttings from 2 of the 3 Tryon wildling trees gave 75 per cent rooting. Cuttings of the third Tryon wildling, which was low in vigor because of wilt infection, failed to root.

To test the value of several growth regulators at different concentrations and under various treatment conditions, the following experiment was set up, with shoots from vigorous trees growing near Asheville, N. C. Cuttings were made in January, 1946, and stored outdoors in moist sand for one month. At this time the cuttings were divided into sets of 6 and, after waxing the upper ends, treated as follows: (1) 100 p.p.m. indoleacetic acid for 24 hours; (2) 40 p.p.m. indolebutyric acid for 24 hours; (3) 100 p.p.m. indolebutyric acid for 24 hours; and (4) no treatment. One-half of each set was dipped in Fermate before planting in flats of vermiculite in the greenhouse. Within one month, 17 per cent of those cuttings given indolebutyric acid at a rate of 40 p.p.m. and 67 per cent of those treated with this acid at a rate of 100 p.p.m. had rooted. All other cuttings had died. There was no apparent rooting benefit from the Fermate treatment. This experiment gives further support to the previous

conclusions regarding the effectiveness of indolebutyric acid, and indicates that a concentration of 100 p.p.m. is much better than 40 p.p.m.

Another experiment was undertaken to test the value of various storage periods and collection times before treatment and planting of cuttings. Stem cuttings were collected in November, 1946, and in January and March, 1947, from 4 resistant trees at Tryon, N. C., a nearby wildling tree, and a wildling from Asheville. These cuttings were stored outdoors in moist sand until April 30, 1947, when they were dug up, soaked for 24 hours in a solution of indolebutyric acid 40 p.p.m., and planted in flats of vermiculite in the greenhouse. At this time the advantage of the 100 p.p.m. concentration had not been determined. Two and one half months after planting, all cuttings stored for 155 days and for 106 days had died. Many of those stored for 51 days were alive but unrooted. At this time they were transferred to a special propagation box similar to that described by Stoutemyer et al. (5), and in 2 weeks the cuttings from 3 of the 4 resistant trees had rooted as follows: 59 per cent, 100 per cent, and 24 per cent; while 57 per cent and 13 per cent of the cuttings from the 2 wildlings had rooted. Cuttings not rooted died within one month. This experiment, together with those already described, shows that individual mimosa trees differ with respect to the rootability of hardwood cuttings.

Greenwood Stem Cuttings

A number of attempts were made to root greenwood cuttings clipped from random field trees in 1945 and 1947, and placed in the propagation box after treatment with hormones. All such cuttings died within 2 weeks without rooting. In another experiment, where rooting of succulent sprouts from root and stem pieces was compared, it was found that only those greenwood cuttings that arose as sprouts from root pieces rooted (7). Much of the variability in rooting from large and small trees and from different parts of the same tree, experienced by several investigators, may be explained on the basis of the nearness of the cutting to the root system.

Root Cuttings

Mimosa has been observed to send up sprouts from cut roots and it seemed possible that propagation from root cuttings would be successful. Roots were collected from 4 resistant trees at Tryon, N. C., a nearby wildling, and a wildling mimosa near Asheville, N. C., in November, 1946, and in January and March, 1947. The roots were divided into cuttings 3 inches long and stored in moist sand outdoors until April 30, 1947, when they were treated with indolebutyric acid at 40 p.p.m. for 24 hours, and planted vertically in the greenhouse in flats of vermiculite, so that about $\frac{1}{4}$ inch of the top end was above the medium. Three months after planting, pieces not rooted were dead. There was no advantage in the longer storage periods, and in some cases they proved detrimental if cuttings became molded during storage. There were great differences in the readiness

with which cuttings rooted from different parent trees. Cuttings from the 4 resistant trees rooted 100 per cent, 50 per cent, 56 per cent, and 89 per cent, respectively, while those from the 2 wildling trees rooted 50 per cent and 42 per cent, respectively.

A comparison was made of the rooting of cuttings in a heated and artificially lighted propagation box and in the greenhouse. Root cuttings were collected on June 11, 1947, from 2 resistant trees and a nearby wildling

TABLE 2.—*Rooting of root cuttings of Albizzia julibrissin after one month in a propagation box and in the greenhouse*

Source	Propagation box		Greenhouse	
	Cuttings rooted		Cuttings rooted	
	Number	*Per cent*	*Number*	*Per cent*
Tryon wildling tree	30	80	29	35
Resistant tree No. 617[a]	22	0	21	0
Resistant tree No. 65	16	56	17	6

[a] This tree is of low vigor.

mimosa at Tryon; after treatment with indolebutyric acid at 40 p.p.m. for 24 hours, they were divided into two lots. One lot was planted in the propagation box and the other in the greenhouse in vermiculite. After one month a much higher percentage of those in the propagation box had rooted than of those in the greenhouse (Table 2). However, after 3 months, more of those cuttings in the greenhouse had rooted, so that the final percentage rooted under the two conditions was nearly the same. The propagation box had the advantage of hastening the rooting of root cuttings.

Inoculation of Cuttings

As a further check on the resistance to wilt of the original resistant selections, rooted stem cuttings from resistant trees and wildling trees were inoculated in April, 1947 with highly virulent strains of the mimosa wilt

TABLE 3.—*Resistance of rooted mimosa cuttings to Fusarium wilt*

Description of cutting	Cuttings inoculated April, 1947		Cuttings inoculated October, 1947	
	Original	Alive April, 1948	Original	Alive April, 1948
	Number	*Number*	*Number*	*Number*
Stem cuttings				
From Tryon resistant trees	8	8	8	8
From Tryon wildling trees	17	0	7	0
From Asheville wildling trees	23	2	8	0
Root cuttings				
From Tryon resistant trees	.....	...	86	86
From Tryon wildling trees	.....	...	22	0
From Asheville wildling trees	.....	...	8	0

fungus, *Fusarium oxysporum* f. *perniciosum*, by methods previously described (6). After 1 year, all of the cuttings from resistant trees were alive, whereas none of the cuttings from wildling mimosas from Tryon survived the wilt (Table 3). Inoculations of additional root and stem cuttings in October, 1947, again indicated that cuttings from apparently resistant trees are resistant to the wilt disease (Table 3).

DISCUSSION

Certain individual mimosa trees are resistant to or immune from the common virulent strains of the mimosa wilt *Fusarium* used in the tests. Although seed from uncontrolled pollination of these trees produced trees, some of which were not resistant, all cuttings rooted from the resistant trees were wilt-resistant. This gives hope that the wilt disease can be alleviated through the replacement of susceptible individuals with resistant clones. However, because of the physiological and morphological variability that has been demonstrated for *F. oxysporum* f. *perniciosum* (4, 6), it is possible that a new form of the fungus may arise in nature, which will attack the present resistant clones.

The mimosa wilt is spreading rapidly, and although many of the resistant trees discovered so far are located outside the known range of the disease, other collections from these outlying areas are susceptible to the disease.

Resistant selections of *Albizzia julibrissin* var. *rosea* and *A. kalkora* may be valuable substitutes for *A. julibrissin*. Other species of *Albizzia* tested, which have some wilt resistance, are tropical or subtropical and not hardy within much of the area where the mimosa is valued.

SUMMARY

In testing certain species of *Albizzia*, mainly *A. julibrissin*, for resistance to Fusarium wilt, 1437 seedlings have been grown from seed collected at various locations from Maryland to Louisiana, and their roots inoculated. Twenty of these trees have survived the disease as long as 8 years, despite repeated inoculations, and in the more recent experiments many more have survived for shorter periods. Fifty-six per cent of the seedlings grown from seed resulting from uncontrolled pollination of the resistant selections were wilt-resistant.

All cuttings rooted from the resistant selections have thus far appeared to be immune from the wilt despite successive inoculations, while rooted cuttings from neighboring nonresistant wildlings became diseased and died following inoculation.

Experiments demonstrated the rooting superiority of root cuttings over stem cuttings, hardwood over greenwood cuttings, the use of the propagation box rather than the greenhouse bench, and no storage rather than prestorage of cuttings in moist sand.

Root cuttings rooted well when planted without hormone treatment; hardwood cuttings rooted well if they were from vigorous young trees, were thicker than 0.3 inch, were waxed at the upper end, and soaked in a suitable hormone solution; greenwood cuttings rooted well and without hormone treatment only if they had sprouted from root cuttings.

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ARSENITE SPRAY INJURY TO GRAPE CANES THROUGH LEAF SCARS

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The application of sodium arsenite to grape vines (*Vitis vinifera* L.), during the dormant season to control black measles is an established practice in the vineyards of California. Spraying the trunk and arms of the Thompson Seedless variety during the late dormant season with sodium arsenite will result in approximately 80 per cent control of this disease.

Periodically there have been reports, particularly from some of the growers in the San Joaquin Valley, that bud injury resulted from this practice. Such injury was especially severe in 1946 and 1947, when an abnormally large number of buds on arsenite sprayed vines failed to produce new growth in the spring. Necrosis of varying degrees was associated with these abortive buds, particularly just below the subtending leaf scar.

Similar symptoms were observed on peach twigs by Haenseler and Martin (2) and also by Poole (3). All concluded the injury was due to the toxic effect of the arsenical content of the spray. Adams (1) made similar observations on peach twigs and arrived at the same conclusion as to the cause of the necrosis. He also noticed, a correlation between severity of premature defoliation and the number of twig lesions that appeared later. On the basis of this observation Adams postulated that the toxic materials of the sprays may gain entrance into the twigs through unhealed leaf scars, or even by way of the leaf traces. Wilson (4) noted that spraying apricot and almond with a dormant spray of monocalcium arsenite in January instead of December markedly reduced bud killing. He concluded that bud killing was due to soluble arsenite gaining entrance into the buds through the unhealed leaf scars.

BUD FAILURE CAUSED BY ARSENITE SPRAY

Spray plots were established during the fall of 1947 in three different Thompson Seedless vineyards in Fresno and Tulare Counties. The soil of two of the vineyards was dry in December and received no water from irrigation or rainfall until February. The soil of the third plot, designated wet was irrigated the first week of December.

The vines were sprayed with a sodium arsenite solution, containing an equivalent of 2¼ lb. arsenic trioxide per 100 gallons of water. Three blocks of vines consisting of 2 rows of 26 or more vines each were sprayed in each of the 3 vineyards on December 15. Additional blocks of vines in the same vineyard were sprayed January 20 and others on February 16. The spray

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was applied to the vines of two of the blocks in each vineyard in such a manner that only the trunks and arms were sprayed. In the third block the canes as well as the arms and trunks were sprayed.

To determine the amount of damage due to the arsenical spray, bud counts in groups of 100 were made on May 7. All of the buds on each individual cane selected at random in treated and nontreated rows of vines were counted. The number of buds which failed to produce any new growth were noted and expressed directly as percentage bud failure. Table 1 gives a summary of these counts for the different spray treatments on wet soil and dry soil plots.

TABLE 1.—*The average percentage of bud failure on sprayed and nonsprayed Thompson Seedless vines (May 7, 1948)*

Plot	Month sprayed	Sprayed trunks and arms only	Nonsprayed	Sprayed trunks, arms and canes	Nonsprayed
	Dec.	18.2		32.5	
A	Jan.	19.7	15.0	23.0	13.0
(dry[a])	Feb.	22.8		34.0	
	Dec.	22.2		17.5	
B	Jan.	22.5	17.5	30.5	17.0
(dry[a])	Feb.	21.7		27.5	
	Dec.	22.0		22.5	
C	Jan.	21.5	17.0	19.7	18.5
(wet[a])	Feb.	18.5		24.5	

[a] Dry—No fall irrigation.
Wet—Fall irrigation.

Whether the vines were sprayed in December, January, or February there appeared to be little, if any, consistent difference in the amount of bud failure.

The slightly higher percentage of bud failure in the rows where the spray was applied only to the trunks and arms over the corresponding checks was probably due to injury caused by arsenite spray accidentally applied to the lower ends of the canes. Bud failure in these rows was most evident at the base of each cane.

On dry soil plots in which the canes were also sprayed the percentage of bud failure in treated rows was considerably greater than in the checks. On the wet soil plot, whether the canes were sprayed or not made no apparent difference in the amount of bud failure, even though in January an examination of leaf scars of canes sprayed in December showed that the traces were necrotic at the point of emergence from the vascular cylinder. However, the necrotic condition did not appear to extend below this point nor to involve the bud immediately above the scar. On the other hand, examination of sprayed buds from the dry soil plots showed necrosis had extended considerably farther below the leaf scar in the vascular cylinder as well as upward to the bud.

HISTOLOGICAL STUDIES OF HEALTHY AND INJURED TISSUES

Nodal segments were collected from the canes of nonsprayed vines on each of the spray dates, and again on May 7. On January 20, February 16, and again on May 7 nodes were collected from the canes of vines previously sprayed. As the nodes from the canes were collected they were stored in Formalin-acetic-alcohol killing and fixing solution. Material for study was sectioned with a sliding microtome, stained in Delafield's Hematoxylin, and mounted in balsam, following standard schedules.

A histological study of the nodes from nonsprayed vines showed that the periderm over the leaf scar was formed prior to leaf fall (Fig. 1, A), and

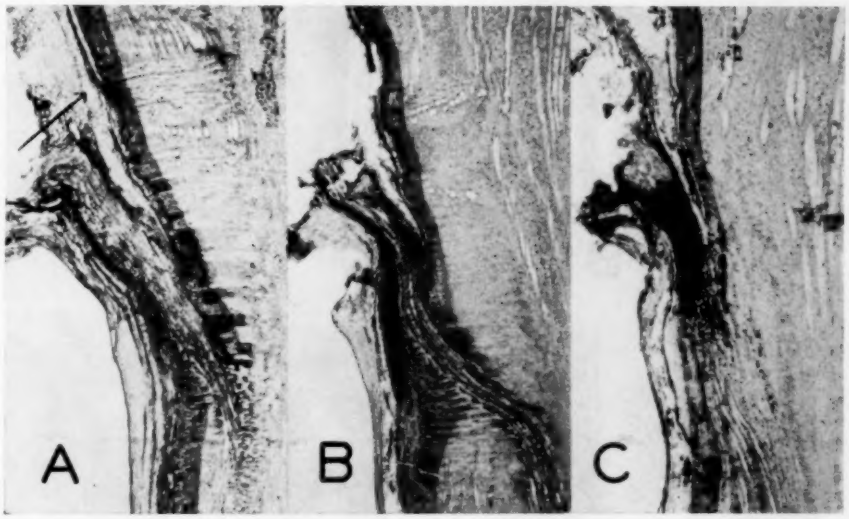

FIG. 1. Photomicrographs of radial sections through median leaf traces of nonsprayed nodes (×10). A. Node collected December 15. Periderm covering the leaf scar above the trace is well developed, (indicated by arrow). The trace is relatively free of gum and the broken ends of the vessels are exposed to the atmosphere. B. Node collected January 20. Wound gum (stained dark) has plugged some of the vessels. Others, however, are still open. C. Node collected February 16. Wound gum has completely plugged all of the vessels of the trace.

that the vessels of the leaf traces were open, and became plugged with wound gum much later. Fig. 1, A, from a radial section of a node collected in December, shows the vessels of the median trace to be relatively free of gum. This condition was apparent in approximately 90 per cent of such nodes examined. In the remaining 10 per cent, many of the vessels were plugged with gum, but in no case were all the vessels of a trace completely plugged. The terminal end of the trace was mechanically broken at leaf fall and the open vessels fully exposed to the atmosphere (Fig. 1, A). A similar section from a node collected in January (Fig. 1, B) shows considerable gum formation in some of the vessels of the trace, though several were still open. Approximately 70 per cent of such nodes examined, showed varying degrees of gum plugging in the traces; about 10 per cent were free

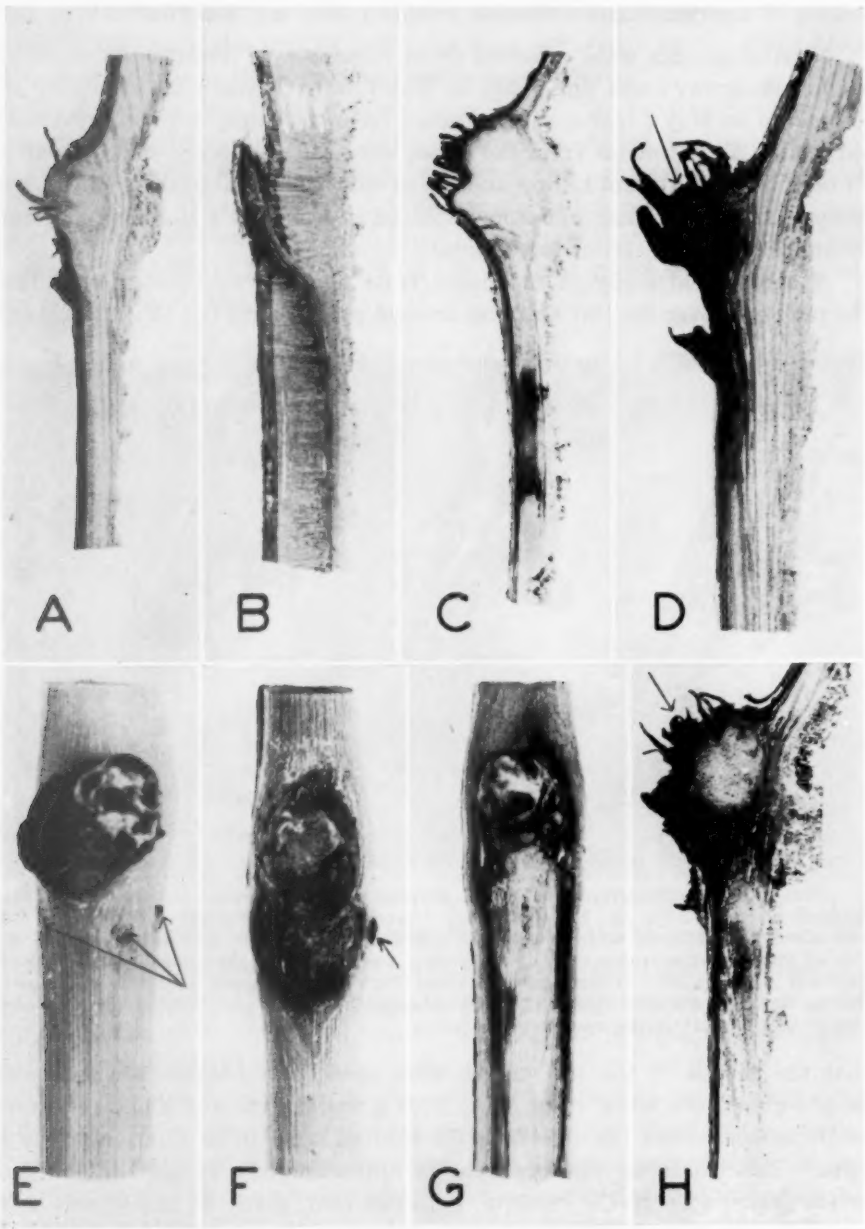

FIG. 2. A, B, C, D and H. Photomicrographs of median radial sections from nodal segments, of Thompson Seedless grape canes. E, F, and G. Photographs of nodal segments with bark removed. A. Section from a nonsprayed node with only the terminal portion of the median trace included (×3). B, C, D and H. Sections from previously sprayed nodes. B. Necrosis (stained dark) has spread downward along the trace and laterally in the medullary ray (×5). C. More advanced stage of necrosis in the xylem tissue (×3). D and H. Upward spread of the necrosis from the leaf trace has killed the bud (×1.5). E. Nonsprayed node with arrows indicating broken ends of leaf traces (×2). F. Necrotic sprayed node. Small necrotic area (indicated by arrow) showing the location of a damaged leaf trace and also showing necrosis below the bud following intake of toxic spray materials through the leaf traces (×2). G. Necrosis of the vascular cylinder which extended to a distance of 4 inches below the bud (×2).

of gum and about 20 per cent were completely plugged. A section from a node collected in February (Fig. 1, C) shows all of the vessels of the trace completely plugged with gum. All such nodes examined were in this condition.

Nodes from vines sprayed with sodium arsenite were necrotic where the trace emerged from the vascular cylinder (Fig. 2, F) of the cane and downward, especially along the trace, in some cases as far as 4 inches below the leaf scar (Fig. 2, B, G). From some traces necrosis also extended laterally into the xylem along some of the medullary rays (Fig. 2, C) and upward into the bud (Fig. 2, D, H).

Since necrosis was found only in the tissues of the nodes from sprayed vines, it was assumed that the injury was due to the entrance of the sodium arsenite into the cane tissues. It appeared from the sequence of nodes examined that the arsenite spray entered the canes through the open and exposed ends of the broken leaf traces, moved downward through the vessels of the trace and radially through some of the medullary rays. Upward movement may have been by diffusion from excessive intake through the leaf trace or by way of the vascular tissue from radial movement in the rays.

SUMMARY

Sodium arsenite applied as a dormant spray to the canes, arms, and trunks of Thompson Seedless vines, (*Vitis vinifera* L.), on dry soil plots, caused appreciable injury as evidenced by sharply defined necrotic areas in the xylem tissue below the leaf scar and bud killing. Vines sprayed only on the arms and trunks had considerably less injury, and it was confined chiefly to lower nodes of the canes. Sprayed vines in a wet soil plot showed only minor xylem injury with little bud killing.

Histological study showed that the leaf scar periderm was formed before leaf fall. Most of the vessels of the leaf traces in scars collected in December after leaf fall were open with no covering over the broken ends and little to no wound gum within the vessels. Some of the vessels in approximately 70 per cent of the leaf traces collected in January were plugged, all of the vessels in about 20 per cent were plugged, and only about 10 per cent showed little or no plugging of the vessels with gum. By February, plugging of the vessels of the leaf scar traces was complete.

A study of damaged buds indicated that the sodium arsenite solution penetrated to the xylem tissue through the vessels of the leaf trace, that necrosis extended downward as far as 4 inches below the leaf scar and also upward into the bud.

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DIFFERENCES IN PYTHIUM INJURY TO CORN SEEDLINGS AT HIGH AND LOW SOIL TEMPERATURES

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A type of injury from *Pythium* sp. to germinating seed corn, different from that which commonly occurs in cold, wet soils (root rot and seed decay), was observed in a recent greenhouse experiment conducted in ordinary compost soil at a temperature range of 20°–24° C. which is very favorable for germination and seedling growth. Seedlings from nearly disease-free kernels that had been wounded by removing a portion of the crown of each, thus exposing the starchy endosperm, were considerably stunted when compared with those from seed similarly injured but which had been dusted with Arasan seed protectant (tetramethyl thiuram disulphide).

Except for their reduced size, the stunted seedlings had few symptoms ordinarily caused by the common seedling blight fungi. Mesocotyl tissues generally were sound and the root systems appeared normal for seedlings of their size. If it had not been for the comparison afforded by the seedlings from the treated seed, the sub-normal conditions of the stunted seedlings (Fig. 1) might have been unnoticed.

Evidence that micro-organisms were involved in the stunting was indicated in the condition of the nontreated kernels still attached to the seedlings. The wounds at the crowns were covered with agglutinized soil and the endosperm and scutellum tissues were more decomposed than in the kernels that had been treated with Arasan. These signs of fungus invasion suggested platings for the isolation of pathogens.

ISOLATIONS AND ARTIFICIAL INOCULATIONS

Platings on water agar from the decayed kernels commonly yielded *Pythium* sp., which readily was isolated in pure culture. The dozen or more isolates obtained all appeared alike in cultural characteristics and might well have been the same species. A single culture, designated C–2, was selected at random from the various isolates and used in inoculation experiments.

METHODS OF INOCULATION

The pathogenicity of *Pythium* sp., culture C–2, was tested on crown-wounded corn kernels by the two following methods of inoculation used by the writer in seed decay studies.

Sand-Culture Method. Week-old cultures, from mycelial transfers, grown in 100 × 15 cc. Petri dishes on 2 per cent potato-dextrose agar are

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FIG. 1. *A*. Healthy corn seedlings grown in compost soil at 20°–24°C. from crown-wounded kernels that had been dusted with Arasan seed disinfectant. *B*. Stunted but otherwise seemingly healthy corn seedlings from nontreated seed. Not shown clearly in the picture is an agglutination of soil at the wounded surfaces of the nontreated seed. This evidence of fungus invasion of the kernels was absent from those that had been treated.

kneaded by hand with autoclaved white sand, mixing the contents from a single dish (agar and fungus) with approximately 250 cc. of sand. A 6-inch earthenware crock then is about half-filled with compost soil after which are added, in successive order, inch-deep layers of autoclaved sand, inoculated sand, and finally, either autoclaved sand or Vermiculite. The corn kernels are planted in the layer of inoculated sand before the covering layer is added.

Fungus-Mat Method. This method is identical with that described above except that the kernels are planted between fungus mats which have been removed intact from the Petri dishes. This more drastic method of inoculation has certain advantages, particularly for tests at low temperatures with cultures of relatively low virulence.

INOCULATION EXPERIMENTS IN WARM AND COLD SOILS

Crown-wounded kernels from a mixture of two, dent corn hybrids, nontreated and dusted with Arasan, were inoculated with *Pythium* sp., culture C–2, by both methods and grown at controlled warm and cold soil temperatures, with one series of controls in naturally infested compost soil and the other in a layer of sterile sand over compost soil.

In the experiment in which the sand-culture method of inoculation was used, the nontreated and Arasan-dusted seed was planted in four 25-kernel replications for each of the following: (1) seed planted in compost soil; (2) seed planted in the inoculated sand; (3) seed planted in a layer of sterile sand over compost soil. This last served as a dual control, for disease from artificial inoculation and also for possible stimulatory effects from the dust.

The inoculations with the fungus-mat method were made in four 15-kernel replications each of nontreated and Arasan-dusted seed.

In the cold soil experiment the crocks first were kept for 14 days in a walk-in refrigerator maintained at 11° C. and then moved to a warm greenhouse (20°–24° C.) to complete germination and seedling growth. The test in the warm soil was made entirely in the 20°–24° C. greenhouse.

RESULTS

The data from the experiment in the warm and cold soils are summarized in table 1 and the more pertinent aspects of the results are illustrated, in part, in figures 2 and 3.

Seed Decay in Cold Soil

Nontreated seed artificially inoculated by either of the two methods resulted in 100 per cent pre-emergence killing (typical seed decay) when kept for 14 days at 11° C. (Fig. 2, A, a). Likewise, nontreated seed planted in compost soil also resulted in no seedling emergence under these conditions. In contrast, seed that had been dusted with Arasan produced good stands of mostly healthy seedlings both in the artificially inoculated sand (Fig. 2,

FIG. 2. Effects of soil temperature on symptoms when crown-wounded corn kernels had been inoculated (fungus-mat method) with *Pythium* sp. *A*. Effects at 11°C.: *a*. pre-emergence killing of nontreated seed, *b*. healthy plants from Arasan dusted seed. *B*. Effects at 20°–24°C.: *a*. stunting without conspicuous lesions on mesocotyls or roots from nontreated seed, *b*. healthy seedlings from kernels treated with Arasan. Results nearly identical with those shown here were obtained following inoculations with the sand-culture method.

A, b) and in naturally infested compost soil. The efficacy of Arasan was impaired perceptibly when the treated kernels were subjected to the drastic fungus-mat method of inoculation, but even with this, the seed treatment resulted in nearly 75 per cent stands of healthy seedlings.

TABLE 1.—*Effects of high and low soil temperatures on disease symptoms caused by Pythium sp. in corn seedlings when nontreated and Arasan-dusted injured seed was planted in naturally infested and artificially inoculated soils and in autoclaved sand*

Source of disease	11°C.			20°–24°C.		
	Stand	Stunted seedlings	Control index[a]	Stand	Stunted seedlings	Control index[a]
	Pct.	*Pct.*		*Pct.*	*Pct.*	
Natural infection (compost soil)						
Nontreated seed	0	.....	0	98.0	59.1	69.0
Arasan-dusted seed	90.0	4.4	88.0	98.0	16.3	90.0
Sand-culture inoculations						
Nontreated seed	0	.....	0	96.0	56.3	69.0
Arasan-dusted seed	87.0	7.0	84.0	100	2.0	98.0
Fungus-mat inoculations						
Nontreated seed	0	.....	0	86.7	100	43.3
Arasan-dusted seed	73.3	0	73.3	93.3	0	93.3
Autoclaved sand (control)						
Nontreated seed	91.0	6.6	88.0	100	7.0	93.3
Arasan-dusted seed	94.0	5.3	91.5	100	2.0	98.0

[a] Theoretical range of control is from 0 (no seedlings emerged) to 100 (perfect stand of all normal-sized plants). Stunted seedlings were given half the numerical values assigned the healthy plants in calculating the control index.

Both nontreated and Arasan-dusted seed produced nearly identical stands of mostly healthy seedlings when the kernels were planted in the noninoculated layer of autoclaved sand in the low temperature experiment. This proved that the seed decay that resulted from artificial inoculation and from sources of natural infection in the compost soil could not be attributed to seed-borne infections.

Stunted Seedlings in Warm Soil

The principal effect of 20°–24° C. when nontreated seed was subjected to inoculation with *Pythium* sp., culture C–2, or when it was planted in naturally infested compost soil, was a pronounced stunting in the seedlings (Fig. 2, B, a and 3, A, a).

The fungus-mat method of inoculation again proved the more drastic in the warm soil with the control index for nontreated seed very significantly lower than for the other sources of infection. This was due partly to a slight amount of pre-emergence killing, but mainly because every seedling from the nontreated seed was stunted.

The results from the sand-culture inoculations more nearly simulated

the disease effects when nontreated seed was exposed to natural infection in the compost soil. Here the control indices were identical. Neither

FIG. 3. *A., a.* Stunted seedlings from nontreated seed, *b.* healthy plants from Arasan dusted seed when crown-wounded corn kernels were planted in compost soil at 20°–24°C.; *B., a.* and *b.* same as *A.* except kernels were planted in a layer of noninoculated, autoclaved sand over the compost soil.

source of infection caused any appreciable amount of pre-emergence killing and both resulted in about two-thirds of the seedlings becoming stunted (Fig. 3, A).

Arasan treatment of seed subjected to artificial inoculation resulted in

almost perfect control of disease at the higher temperature. Some stunting (16 per cent) recorded for treated seed planted in the compost soil might have been due to the presence of more virulent forms of *Pythium* in the naturally infested soil than the pure culture used in the artificial inoculations.

Only a few stunted seedlings (7 per cent) were observed among those from kernels that had germinated in the layer of autoclaved sand (Fig. 3, B). This demonstrated rather conclusively that the main stunting effects in these experiments were caused by the soil fungi and not by seed-borne parasites. It is very likely that the 7 per cent of stunting mentioned was caused by seed infections because platings of the seed disclosed some seed infections with miscellaneous fungi some of which probably were capable of causing stunting at the higher temperatures.

DISCUSSION

The belief commonly held that disease-free seed corn is not attacked by *Pythium* or other seed-decay types of fungi, except in cold wet soils, is disproved in these experiments. Severe stunting in seedlings from nontreated, crown-wounded kernels was demonstrated at soil temperatures very favorable for germination and seedling growth (20°–24°C.) in naturally infested soil and also following pure culture inoculations with *Pythium* sp., culture C-2. This fungus, originally isolated from a stunted seedling grown in warm soil, was proved similar to the seed-decay types in artificial inoculations in cold, wet soils where it caused 100 per cent pre-emergence killing in nontreated seed.

Whether the stunting caused by *Pythium* sp., culture C–2, in warm soils is typical of what might be expected from other seed-decay fungi under similar circumstances has not been investigated. In view of the unspecialized type of parasitism involved in the destruction of endosperm and scutellum tissues, it would seem probable that other seed-decay fungi would behave similarly. The artificial methods of inoculation described herein provide a ready means for a study of this question, and also for a much-needed investigation of soil fungi in general, with respect to their disease potentialities in germinating seed corn.

The excellent protection against stunting in warm soil afforded by Arasan seed treatment in the present studies indicates that benefits from seed treatment with this and other efficacious dusts occur in most field plantings, regardless of how favorable the temperatures during the germination period. Despite improved methods of processing seed corn to reduce the amount of mechanical damage, many injured kernels may still be found in most commercial seed lots.

The present study illustrates an advantage of investigating the complicated corn seedling disease problem in singling out an individual variable from the many involved in the complex and then studying that one in "accentuated" tests where every kernel, theoretically at least, responds

alike when subjected to experiment in a controlled environment. The disease in the warm soil described herein would not have been easily detected, much less interpreted correctly, in experiments with ordinary samples of seed.

SUMMARY

Stunting in corn seedlings without conspicuous lesions on mesocotyls or roots, first observed when nontreated nearly disease-free kernels with crown wounds were planted in warm (20°–24°C), compost soil, was demonstrated at this temperature following pure culture inoculations with *Pythium* sp. This fungus, originally isolated from a naturally infected seedling grown in warm soil, caused 100 per cent seed decay in inoculations of nontreated seed at 11°C.

Arasan seed treatment afforded excellent control against stunting in both naturally infested and artificially inoculated soils. The benefits demonstrated in the warm soil are an advantage of corn seed treatment heretofore unrecognized.

Two methods of pure culture inoculation with *Pythium* sp. are described.

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EVIDENCE OF THE NON-EXISTENCE OF PHYSIOLOGICAL RACES IN CRONARTIUM RIBICOLA

GLENN GARDNER HAHN[1]

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Apropos of Mielke's (6) fairly recent and brief discussion of physiological races or strains of white-pine blister rust, which is caused by *Cronartium ribicola* A. Fisch., it is desirable at this time to present a summary of certain accumulated data relating to the Viking red currant (2). Its long history of freedom from the establishment of the pine parasite upon its leaves, serves to support the view held by some pathologists that there is only one race in *C. ribicola*.

Although the new varietal name, Viking (2), was published in the mid-30's, the red currant itself is not new. When the rust-immune Viking (syn. Rød Hollandsk Druerips, Holländische Rote), with probable other synonyms, was introduced specifically for blister-rust investigations in the early 1930's into the United States from Norway, it was deemed advisable to report the foreign introduction under a new name in order that it would not be confused with our domestic rust-susceptible red currant variety, The American Red Dutch (2). Viking, a *Ribes petraeum* Wulf. hybrid, actually is a very old variety and has been known in the horticultural literature for at least a century and probably very much longer. Under the name, Rød Holländsk Druerips (2), the currant has been grown in Scandinavia for many years in gardens and commercial plantations; as Holländische Rote it has been cultivated extensively on the European Continent as well, particularly in Holland, Germany, and Switzerland. Accordingly, the variety has been exposed to blister-rust infection over wide areas in western Europe for many years. There are no records (5, p. 345), however, of *Cronartium ribicola* having been observed upon its leaves. The late eminent forest pathologist, Tubeuf (7), working independently, tested Holländische Rote at Munich, Germany, and reported it to be immune from *C. ribicola*. The following decade the writer reported stock of Holländische Rote from the Grafrath Experiment Station, Munich, as being, as Tubeuf had recorded, immune from the rust (5). Field tests later showed the German introduction to be identical morphologically with the Viking stock from Norway.

Before the writer undertook American investigations, he tested Viking brought from Norway into Great Britain with *Cronartium ribicola* collected in Scotland and found the red currant to be immune (1; 2, p. 9; 5, p. 343). In this respect in accordance with the writer's concept of the term im-

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munity, it implies an invasion of Viking leaves by germ tubes of *C. ribicola* at certain stages of leaf development, but as previously indicated the infectious agent is unable to establish itself (5, p. 343). Subsequently the writer demonstrated under conditions of supervised testing in the United States that the introduced variety was immune when exposed to natural infection of *C. ribicola* in the East in New England (Maine, New Hampshire, Massachusetts, and Connecticut) and in New York State, and in the West in Oregon (3).

In Canada, similar stock (3, p. 861) of the variety provided by the United States Department of Agriculture was tested independently by Canadian pathologists and horticulturists at the Central Experimental Farm, Ottawa. These workers were unable to find any establishment of *Cronartium ribicola* on leaves of Viking although nearby plants of varieties of *Ribes nigrum* L. became heavily infected with the rust (5, p. 347).

In addition to these supervised field experiments, large plantations of Viking, which was also shown by the writer to be nearly homozygous for rust resistance (4), were established for horticultural testing by Professor W. H. Alderman in 1936 in Minnesota at the North Central Experimental Station at Grand Rapids and at the Fruit Breeding Farm of the University of Minnesota near Excelsior. This stock, like that in the foregoing tests mentioned, had been propagated from cuttings introduced from Norway (3, p. 861) and distributed by the United States Department of Agriculture. According to information transmitted by Professor Alderman, the currant has done very well and has been free of rust in white-pine (*Pinus strobus* L.) territory where Viking, with the approval of the local blister-rust authorities, is the only red currant grown.

On the basis of experience with cereal rusts in which different races are known to occur, it might be anticipated that more than one race of *Cronartium ribicola* exists, and, if so, one of them might be capable of becoming established upon Viking. However, results with the variety in North America together with observations and tests on it in Europe have not revealed the establishment of blister rust upon its leaves. If there are races of *C. ribicola*, it has been suggested that Viking may react the same to all of them. Moreover, if different physiologic races of the pine parasite actually exist, experience with Viking over many years in Europe and North America has not indicated their presence.

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PHYTOPATHOLOGICAL NOTES

The Dissemination of Xanthomonas malvacearum by Irrigation Water.—Wind-blown rain and droplets of splashed water from fog and dew have long been recognized as disseminating agents in the spread of angular leaf spot of cotton.[1,2] Evidence is presented here which indicates that surface irrigation water may also act as a disseminating agent.

During the course of a seed treatment experiment at Sacaton, Arizona, in 1938, two plots of Pima cotton seedlings, *Gossypium barbadense* L., became severely affected with angular leaf spot. The disease appeared shortly after the first flood irrigation, which was applied May 2. A previous disease survey of these plots had shown no angular leaf spot, although a plot lying 15 feet above them in the same irrigation border had approximately 10 per cent infected plants. By May 19, the two plots that at first had been disease-free had 67.5 per cent of 891 seedlings infected in one instance and 46.0 per cent of 1480 seedlings infected in the other.

As there had been no rain to account for the spread of the bacterium, the irrigation water was believed to be the disseminating agent. To test this hypothesis, a nearby disease-free planting of Pima cotton seedlings was divided into three parts, designated plots 1, 2, and 3, and bordered so that each could be flood irrigated independently. On May 23, irrigation water was applied to plot 2 after it had passed through plots that were infested with angular leaf spot. The distance between the diseased plot and the disease-free plot was about 15 feet. Plots 1 and 3 were irrigated at the same time with water from lateral ditches that had no opportunity to contact cotton seedlings. A census on June 14, showed 44.2 per cent infection in 486 seedlings in plot 2, whereas plots 1 and 3 with a combined total of 889 seedlings remained free of the disease. In this instance also there had been no rain. Thus the results were in agreement with the earlier observations.[3]

In 1943 both surface irrigation and sprinkling were effective in disseminating *Xanthomonas malvacearum* (E. F. Sm.) Dowson. This was demonstrated during studies on the winter carry-over of angular leaf spot. A previously diseased crop of the upland variety Acala (*Gossypium hirsutum* L.) was used as a source of primary infection, while a disease-free planting of the American-Egyptian variety S × P (*G. barbadense* L.) was used to determine the spread of the disease. Both are susceptible to angular leaf spot and the seedlings of each may be readily distinguished upon close examination.

The test was conducted as follows: Acala bolls were collected in Feb-

[1] Faulwetter, R. C. Dissemination of the angular leafspot of cotton. Jour. Agr. Res. [U.S.] 8: 457–475. 1917.

[2] Faulwetter, R. C. Wind-blown rain, a factor in disease dissemination. Jour. Agr. Res. [U.S.] 10: 639–648. 1917.

[3] Reference was made to this experiment in the following report: King, C. J. and R. B. Parker. Angular leaf spot of cotton in the irrigated valleys of Arizona and New Mexico. U. S. Dept. Agr., Pl. Dis. Reptr. 23:32. 1939.

ruary from a field that had had angular leaf spot the previous season. In March the bolls were spread across the upper end of a plot of land that had not been cropped with cotton for several years. The soil was plowed, irrigated, and disked; and on March 23 it was planted with seed known to be free of *Xanthomonas malvacearum*. A good stand was obtained

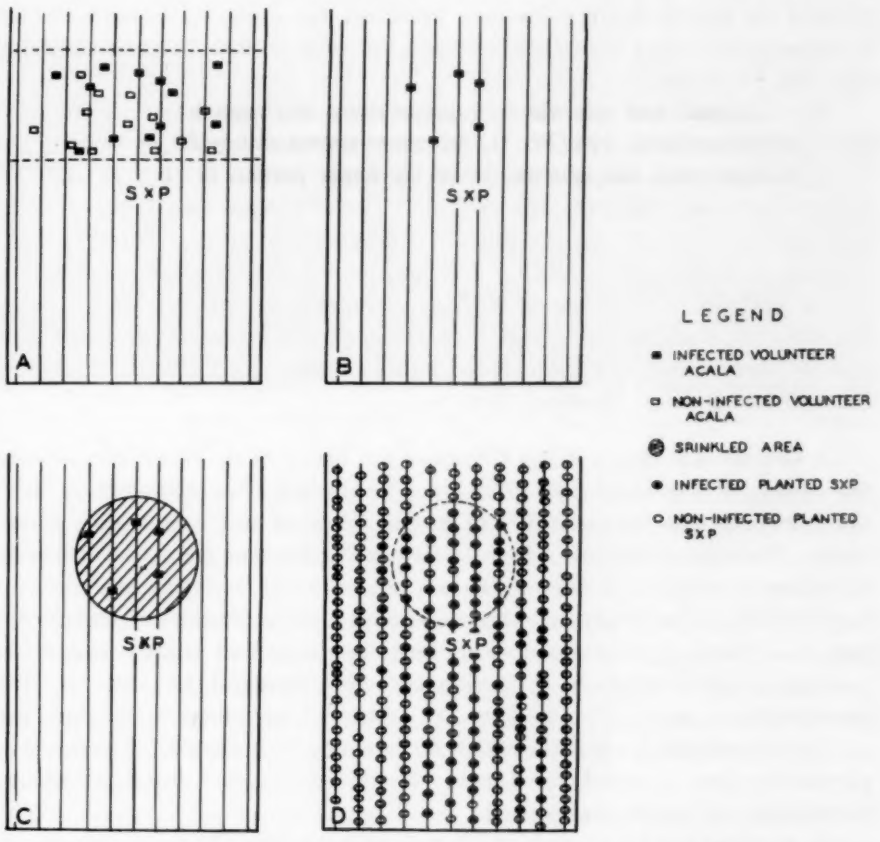

FIG. 1. Maps showing the spread of angular leaf spot from diseased volunteer Acala seedlings to disease-free planted S × P seedlings. A. Infected and non-infected Acala seedlings as they occurred among the planted rows of S × P. B. Infected Acala seedlings remaining in the S × P seed rows after cultivation. C. Sprinkled area in which a majority of the S × P seedlings became infected. D. Thinned stand of S × P seedlings showing infected and non-infected plants following flood irrigation.

from the planted seed and a small percentage of the buried Acala bolls produced seedlings. The plot was mapped on April 27 (Fig. 1, A). Approximately one-half of the clumps of Acala seedlings had angular leaf spot, but no infection was found in the S × P seedlings. A cultivation on May 11 removed all of the clumps of Acala seedlings except those in line with the seed rows. Five clumps with infected seedlings remained (Fig. 1, B). On May 12, a rotating lawn sprinkler was placed in the

portion of the plot in which the diseased Acala seedlings were located. Within 10 days, a majority of the S × P seedlings within the sprinkled area had angular leaf spot lesions. The affected area of the plot coincided with the sprinkled area (Fig. 1, C), while all the seedlings outside remained disease-free. On May 22, the entire plot was flood irrigated, the seedlings being partially submerged for about 1 hour. The stand was thinned to approximately 15-inch spacings on June 2, without regard to angular leaf spot infection, and the entire plot was then mapped for diseased seedlings.

Angular leaf spot was then found below and immediately to the sides of the sprinkled area (Fig. 1, D) where approximately 50 per cent of the thinned stand was affected, while the upper portion of the plot and the outside rows remained disease free. Thus the disease had spread in the same general direction as the flow of the irrigation water. Infected seedlings showed that the pathogen had been carried in two instances to the end of the plot, a distance of 25 feet. Again there was no rain between the time of planting and final inspection of the plot.—C. J. KING, late Senior Agronomist, and L. A. BRINKERHOFF, Associate Pathologist, United States Field Station, Sacaton, Arizona.

A Greenhouse Bench Moist Chamber for Large Scale Inoculations.[1]—In the course of a breeding program, it is frequently desirable to test large numbers of plants for resistance to specific diseases under controlled conditions. For many diseases, a high humidity for a given period is necessary to induce infection. However, the desired humidity is frequently difficult to achieve in an ordinary greenhouse without a moist chamber. During the past two years, a moist chamber of large capacity and simple design has been in operation at the U. S. Regional Pasture Research Laboratory. The chamber has a capacity of 26 flats (12 × 24-inch) or 400 pots (4-inch) and can be constructed for less than $100.00. It may be made either portable or permanent over a greenhouse bench. The moist chamber and humidifying mechanism are shown in figure 1.

In erecting the framework, 2 × 2-inch uprights were fastened at each inside corner of the bench. Wooden braces were secured to the uprights and wires were stretched the length of the bench and attached to the uprights. Another wire was fastened to the greenhouse frame to support the peak of the roof for the chamber. Plastic shower curtain material, clear Parafilm,[2] was then spread over the wires to form a canopy. Strips 15 feet long and 3 feet wide were lapped about two inches at the borders and were long enough so that the ends reached below the sides of the bench. Each strip

[1] Contribution No. 88 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Dept. of Agriculture, State College, Pa., in cooperation with the northeastern States.

[2] Parafilm was obtained from the Para Mfg. Company, Inc., 500 Central Avenue, Newark 7, New Jersey.

thus formed a panel that was easily folded back to afford access to plants at any place in the chamber. The ends of the chamber were fashioned of the same material, cut to fit and tacked to the wooden brace between the uprights. The upper portions were pinned to the nearest canopy panel. At one end of the chamber a hole was cut in the Parafilm to allow introduction of moisture from the humidifier.

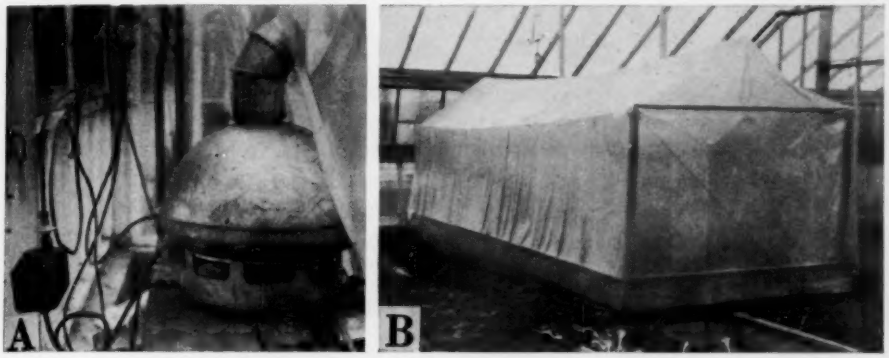

FIG. 1. A. Close-up view of humidifying apparatus. B. Moist chamber erected over a greenhouse bench.

A Model 42 humidifier manufactured by the Standard Engineering Works, Pawtucket, Rhode Island, was used. The unit draws water up the outer surface of an enclosed cone rapidly rotated by an electric motor and throws it against the vanes of a circular grid where it is broken into a fine mist and blown into the chamber through an adjustable elbow. A float valve attached to a water line regulates the water level in the reservoir of the humidifier. At present, the unit is operated satisfactorily by an automatic time clock that turns the humidifier on and off 15 minutes out of every hour from 7:00 a.m. to 7:00 p.m. It has not been found necessary to operate the unit at night.

The artificial fog is circulated to all parts of the chamber within one minute after the humidifying unit starts to operate. The moisture particles are small and settle slowly and uniformly, thus covering leaves of plants within the chamber with a film of moisture resembling dew. The Parafilm canopy admits sufficient light to prevent etiolation of plants even when kept in the chamber for 10 to 14 days. It is mildewproof, resists tearing, and to date shows no sign of deteriorating. The chamber has operated satisfactorily in large-scale inoculation tests with *Sclerotinia trifoliorum* on forage legumes. Excellent infection has also been obtained with such foliar diseases as *Puccinia coronata* on *Festuca elatior* and *Stemphylium sarcinaeforme* on *Trifolium pratense*.—K. W. KREITLOW, U. S. Regional Pasture Research Laboratory, State College, Pennsylvania.

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